A NEW TECHNIC FOR THE MEASUREMENT OF AVERAGE SKIN TEMPERATURE OVER SURFACES OF THE BODY AND THE CHANGES OF SKIN TEMPERATURE DURING EXERCISE [1]

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SIX FIGURES

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The earliest measurements of skin temperature were made by Davy in 1814, by holding the bulb of a thermometer against the skin. Although obviously considerable errors are introduced, this method is still employed and perhaps is of sufficient accuracy in cases where interest centers in changes in the temperature of a particular area and where the absolute value of the temperature is immaterial. The introduction of thermocouples brought a great improvement in accuracy and many measurements of skin temperature at different parts of the body have been made by this method by Benedict, Miles and Johnson, Benedict and Parmenter, Talbot, Cobet and many others. A review of the literature is given by Cobet ('26). Their apparatus is fully described in the papers referred to. Aldrich ('32) pointed out that the type of construction of the thermocouples used in the majority of these experiments introduced a fundamental error. One or more junctions of the thermocouple were pressed against the skin or clothes by an insulating holder. Behind the junctions was a pad of cotton wool or other heat insulating material. The temperature of the skin is such that there is an equilibrium

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between the heat reaching it from the interior and that lost by convection, radiation and evaporation. The application of a thermocouple of the type described necessarily interferes with the heat loss from the area with which it is in contact, and there is a consequent rise in temperature of an undetermined amount. It is thus essential that the portion of the thermocouple that is in contact with the skin should have as small a heat capacity as possible and have a minimum effect upon loss of heat. Thermocouples of fine wire without backing of insulating material other than a fine silk thread have been used by Aldrich and fulfill the conditions well. The thermocouples used by McClure and Sauer ('15) also were satisfactory in this respect, but this source of error seems to have been overlooked in more recent work.

These experiments have revealed that there are very considerable differences, amounting to as much as 10°C. between different parts of the surface of the body when it is in a cold environment. When studies are being made of the changes in skin temperature from individual to individual and in the same individual under different conditions, it is the average skin temperature over large areas of surface or over the whole body that is of significance. Also it is recognized that the average skin temperature over the body determines the heat loss to a great extent and a knowledge of this would be a valuable index to heat loss and metabolism. The great range of temperature in the different parts of the skin surface renders the estimation of such an average skin temperature by the use of thermocouples very difficult. The method to be described has been designed to give directly an average of the temperature over an extended area, such as the trunk, with the least disturbance of the conditions existing before the measurement is made. It is adapted to give continuous records of the temperature in conditions such as in the measurement of the skin temperature under clothing, during exercise and sleep, in which the thermocouple methods are inapplicable. Where interest centers in local changes of temperature of small areas, as in examination of the sympathetic

vasoconstrictor activity (Morton and Scott, '30), it has no superiority over other methods.

For the thermocouple, a resistance thermometer is substituted. This type of thermometer was used by G. N. Stewart (1891) for measurments of skin temperature. The principle has been in use at the Russell Sage Institute and thermometers have been described recently by Soderstrom ('33). The thermometers here described differ greatly, however, from those employed previously.

Nickel has a large temperature coefficient of resistance of about 0.4 per cent der degree centigrade. It was found possible to fix a continuous length of fine nickel wire, of no. 40 gauge, enameled and cotton covered, to cloth by sewing. The nickel wire was placed in the lower spool of a sewing machine while ordinary thread was used above. When proper adjustment of the tension of the thread was attained, the nickel wire was held to one side of the cloth without serious kinking which would tend to cause breakage of the wire. In this way wire was fixed in lines about ½ inch apart up and down a piece of cloth shaped to fit as a jacket around the trunk of the subject. The ends of the wire were anchored securely by sewing to the cloth and connected to flexible leads. In order that the wire might be held closely in contact with the skin the jacket was fastened at the back by adjustable elastic tapes. Adjustable shoulder straps made the vest adaptable to various subjects. In all there were some 40 feet of wire in the jacket and the resistance was of the order of 200 ohms. Its resistance therefore changed by about 1 ohm for every degree centigrade.

Similar 'jackets' were made using both medium weight cloth, such as is used for nurses' uniforms, and single weight gauze; the latter being designed to give least interference with the evaporation and loss of heat from the bare skin. A stocking similarly constructed, to measure the skin temperature over the leg has also been used, and the method of construction can be used to make a thermometer adapted to almost any area desired.

To record the changes in resistance, and therefore of temperature, the usual arrangement of the Wheatstone network was used, shown in the diagram (fig. 1). Coils were wound of constantan wire, which does not change its resistance with temperature, and a special box was made up, but any form of resistance bridge available might be used. The two 'ratio arms' are equal, of 300 ohms each. The other two arms of the bridge that are being compared are alternatively, by the throw of a double pole, double throw, switch, either the jacket

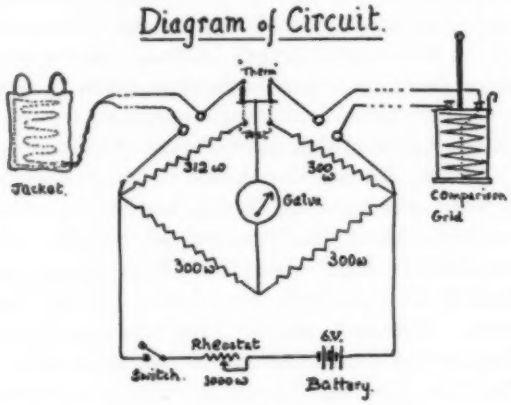

Figure 1

resistance and a comparison resistance, or with the switch in the 'test' position, two fixed constantan coils that differ in resistance by a known amount, say 12 ohms. The comparison resistance consists in the first place of a constantan coil of resistance such that it is equal to that of the jacket at some standard temperature, say 24°C. With the jacket at that temperature there is then no deflection of the galvanometer. At any other temperature the nickel wire in the jacket has a different resistance, the balance of the bridge is disturbed, and the deflection of the galvanometer is very closely proportional to the change in temperature of the jacket.

In use the switch is first put to the 'test' position and a deflection results which is equivalent always to the deflection that would be produced by a certain difference of temperature of the jacket above the zero temperature (the 24°C. mentioned). The value of this difference of temperature was determined by a calibration experiment in which the jacket in a metal container was immersed in water baths of known temperatures. For instance, it was found that 10°C. difference of temperature in the water baths produced a change in the deflection of, say, fifteen divisions of the scale of the galvanometer, while in the 'test' position the deflection was twelve divisions. The test deflection therefore corresponded to $12/15 \times 10 = 8$°C. If then in any subsequent experiment the deflection corresponding to the temperature of the jacket is 'x' divisions, while the test deflection is 't' divisions, the jacket must have been at a temperature $x/t \times 8$°C. above the temperature level. The latter temperature is found from the same calibration experiment, since from the deflections at two different temperatures it is easily deduced at what temperature the deflection would be zero. Thus an equation is found by which deflection may be turned into temperature. In the case cited above it would be:

$$T = 24.0° + x/s \times 8°C.$$

The advantage of this system of a 'test' deflection is that it renders the method independent of the particular galvanometer used and of the current in the bridge. If the sensitivity of the galvanometer or the voltage of the battery supplying the current change in the course of an experiment, as long as test deflections are taken before and after the change, no new calibration is needed.

It is obvious that by adjustment of the current in the bridge so that the test deflection is some standard value the temperatures may be read off the scale directly, as for instance, if the test deflection 's' was made to be always eight divisions of the scale in the example above. Each division would then represent 1 degree. If 's' were made four divi-

sions, each division would be equivalent to 2 degrees. The sensitivity can thus be conveniently adjusted to any approximate value. Resistance thermometry has the great advantage over the use of thermocouples in that the sensitivity can be increased merely by increase in the current through the bridge supplied by the battery. Thermocouple deflections can be increased only by the use of a more sensitive galvanometer.

The galvanometer used in the majority of experiments was of the 'thread recorder' type made by the Cambridge and Paul Instrument Company. This records the deflection upon a chart on a moving drum at intervals of 1 minute. It is fitted with a switching mechanism so that records of two different currents may be, if desired, recorded simultaneously. Any other type of galvanometer of sufficient sensitivity, recording or otherwise, may, of course, be used. With the particular arrangement used a sensitivity of about 0.02°C. was obtained when a current of about 0.01 ampere flowed in the bridge In view of the physiological variations no advantage would be gained by increasing the sensitivity. It may easily be shown that the heating effect of a current of this magnitude in the wire of the jacket introduces a negligible error in the determination of the skin temperature.

For the estimation of heat loss from the body, it is not the absolute level of skin temperature but rather the 'excess temperature' between the skin temperature and the surroundings that determines the heat loss. It has often been pointed out that the heat loss by radiation and convection is proportional to the excess temperature. In order that the excess temperature might be recorded directly, a grid of the nickel wire of the same length as in the jacket was sewn in a piece of the same material and fixed around a wooden frame. The wire lies on the inside of the cube thus formed, its two ends being brought out to leads connected to the bridge in place of the fixed constantan comparison resistance. Adjustment of the length of nickel wire in this comparison grid, so constructed, was carefully made until when jacket and comparison grid were at the same temperature there was no deflection

of the galvanometer. The subsequent deflections then indicated directly the difference of temperature between the skin of the subject and the temperature of the air at the place where the grid is placed. When the subject is lying in bed, the latter is hung by the hooks shown to the rail of the bed. Since the construction of the comparison grid is similar to that of the jacket, fluctuations in the room temperature will affect the two to the same extent and with about the same lag in time. Thus a record of the excess temperature may be made over a long period, irrespective of fluctuations of room temperature, as in the case of the skin temperature of a sleeping

Figure 2

subject through the night. The comparison grid is shown to the right hand side of the photograph of the apparatus (fig. 2).

It was found that from 10 to 15 minutes was amply sufficient for the temperature of the jacket to have reached the maximum temperature, i.e., that of the skin. After that point the temperature recorded would remain constant to well within 0.1°C. provided there were no physiological changes in the subject. Draughts of air, the proximity of any radiating body such as a person or a strong light, any sudden move-

ments of the patient, or the onset of sweating show themselves immediately. An example of the constancy of the temperature is shown in figure 3, from an actual record of the galvanometer.

Studies are being made of the level of skin temperature over the chest in normal and febrile subjects of different sizes and ages under basal conditions. For this purpose it is desirable that the area of skin measured should not extend to the part of the back that is in contact with the bed, as movements here would alter the heat loss of this area and thus the recorded temperatures. Accordingly the 'jackets' are here simply rectangular pieces of gauze sewn with wire as described. The sizes are such that when tied around the trunk of the subject the area covered by the nickel wire ends just posterior to the line from shoulders to hips.

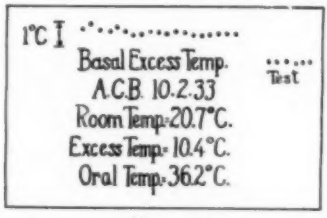

Figure 3

By the aid of this technic studies are being made of the mechanism of heat loss and of the heat regulating mechanism of the body. These are not yet complete, but as an example of the use of the method some studies of the skin temperature during and after muscular exercise are here reported.

Benedict ('25) and Benedict and Parmenter ('28) studied the effect upon the skin temperature at a number of points of the body of exercise such as outdoor walking, running and work upon a treadmill in the laboratory. They used thermocouples and had no means of recording the skin temperatures during the performance of the muscular work. Readings taken before and after the exercise showed some inconsistencies but they concluded that in general "there was a distinct drop in average peripheral temperature as a result

of muscular activity for five minutes, in spite of the fact that the metabolism is greatly increased and a greater heat loss would be expected." They suggest that this might be due to a vasoconstriction of the peripheral vessels to transport blood from the periphery to the muscles. Talbot ('31) came to the same conclusion with children as subjects. A contrary rise of temperature was sometimes found in the case of the hands and cheek, although the temperature of the forehead always fell markedly.

The technic described offered a means of observing the changes of skin temperature during the exercise. The work was performed and measured upon a magnetically braked bicycle ergometer. The subject wearing a gauze resistance jacket over the bare chest sat at rest in the saddle for 10 or more minutes until the skin temperature was at a constant level. The rectal temperature was taken during this period. He then began pedaling, at the point marked A on the records, against various loads and at measured rates, for a period of 10 minutes, ceasing at the point C on the records. Except for an interruption to take the rectal temperature after the exercise, he then remained at rest on the bicycle until the skin temperature was again normal.

Some typical records for increasing amounts of work are shown in figure 4. These are actual charts made by the recording galvanometer, the dots being merely emphasized and joined by a line for sake of clearness. There are of course inconsistencies in individual cases but the general trend of the curves may be described as follows.

Immediately upon the commencement of the work, at the point marked A on the charts, a rise in skin temperature was evident. This amounted to 0.2° or 0.3°C. and occurred in less than a minute after the start of the exercise. In some cases it was observed, though not seen upon the record, since the galvanometer marks only the deflection at the end of each minute. The initial rise was followed by a period in which the temperature remained practically constant until after some 6 to 8 minutes there began usually a fall in skin

temperature at a quite definite point marked B in the records. The work ceased at the point C, upon which there was in most cases immediate acceleration of the fall of temperature. After some 5 to 10 minutes the lowest temperature was reached, considerably below that which prevailed before the exercise. The amount of this total fall is seen to increase

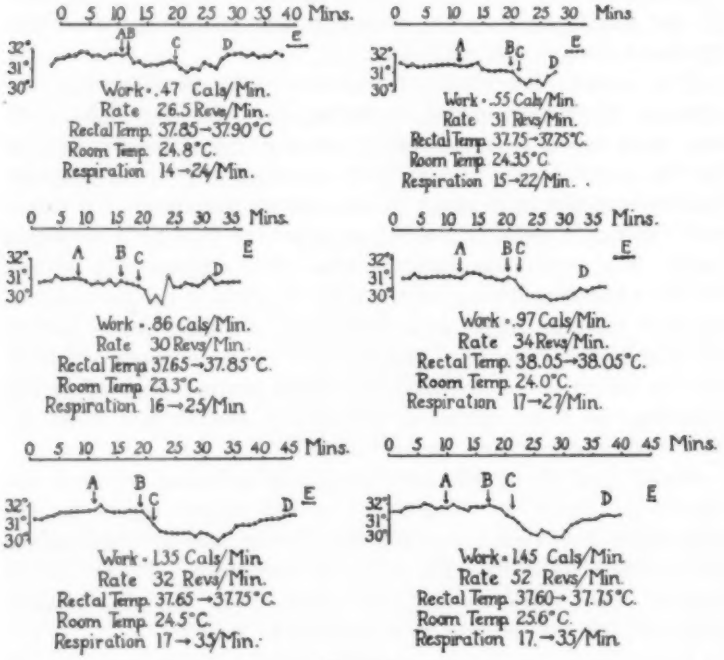
0 5 10 15 20 25 30 35 40 Mins.
32°
31°
30°
A B C D E
Work = .47 Cals/Min.
Rate 26.5 Revs/Min.
Rectal Temp. 37.85→37.90°C.
Room Temp. 24.8°C.
Respiration 14→24/Min.
0 5 10 15 20 25 30 Mins.
A B C D E
Work = .55 Cals/Min.
Rate 31 Revs/Min.
Rectal Temp. 37.75→37.75°C.
Room Temp. 24.35°C.
Respiration 15→22/Min.
0 5 10 15 20 25 30 35 Mins.
A B C D E
Work = .86 Cals/Min.
Rate 30 Revs/Min.
Rectal Temp. 37.65→37.85°C.
Room Temp. 23.3°C.
Respiration 16→25/Min.
0 5 10 15 20 25 30 35 Mins.
A B C D E
Work = .97 Cals/Min.
Rate 34 Revs/Min.
Rectal Temp. 38.05→38.05°C.
Room Temp. 24.0°C.
Respiration 17→27/Min.
0 5 10 15 20 25 30 35 40 45 Mins.
A B C D E
Work = 1.35 Cals/Min.
Rate 32 Revs/Min.
Rectal Temp. 37.65→37.75°C.
Room Temp. 24.5°C.
Respiration 17→35/Min.
0 5 10 15 20 25 30 35 40 45 Mins.
A B C D E
Work = 1.45 Cals/Min.
Rate 52 Revs/Min.
Rectal Temp. 37.60→37.75°C.
Room Temp. 25.6°C.
Respiration 17→35/Min.

Figure 4

with the severity of the exercise, from 0.08°C. for the lightest load to nearly 2°C. for the heaviest. A subjective sensation of sweating, apparent first upon the forehead, coincided with the point B, and there was a feeling of cold on the skin after the cessation of work at C. The temperature then gradually rose until some 15 to 20 minutes after the end of exercise the temperature had reached constancy at a level equal to or

slightly lower than the original level before exercise. The points marked E on the charts are the 'test deflections' explained earlier in the paper. As is well known in the physiology of muscular exercise, there was in general a rise of rectal temperature which was greater the greater the work done. For the heaviest loads it amounted to 0.2°. The respiration rate increased proportionally to the work done.

The natural interpretation of these recorded changes in skin temperature is that the initial rise is due to the increased circulation upon starting the work, which brings up more heat to the surface of the body. A similar rise in the skin temperature has been recorded, in the case of subjects lying quietly on a bed, following the smallest movement or even tension in the muscles. The fall of temperature starting at B can evidently be attributed to an increase in the evaporation of water from the skin, produced by a stimulated activity of the sweat glands starting at this point. Thus when the exercise ceases at C, this evaporation continues to cool the skin, and since the circulation quickly returns to normal there is no compensating increase in heat brought to the skin to slow the fall of temperature. The total drop of temperature produced by the evaporation would naturally depend upon the total amount of sweat to be evaporated, and thus increased with the severity of the exercise. Finally sweating has ceased and as soon as the remaining water has been evaporated and the skin dried the temperature returns to normal. The tendency to stay at a slightly lower level after the exercise from that preceding it may be evidence of a compensating decrease in the circulation after the period of extra load upon the heart or may merely indicate that drying is not complete for a long time.

Confirmation of the view that the decrease in skin temperature during exercise is due to sweating is furnished by some experiments in which the gauze resistance jacket was replaced by one of 'uniform cloth.' The effect of this is to allow the extra heat during exercise to accumulate at the skin and to delay the effects of evaporation. A typical curve is shown

in figure 5. The phases that have been described are now much more clearly demarked. The initial rise of temperature is much greater, amounting to 1.5°C.; the onset of sweating at B is plainly seen and the increase in the slope at C when work ceases. The final drying of the skin and rise of temperature to the original level is, as would be expected, much slower.

It was thought of interest to compare the changes in skin temperature on the leg of the subject when riding the bicycle, as here in addition to the increased circulation there is considerable work done by the underlying muscles. A gauze

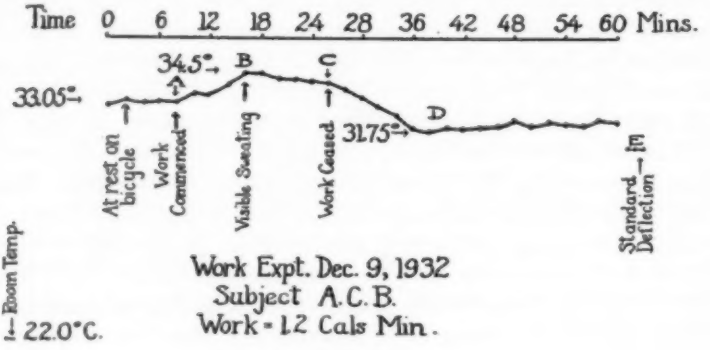

Figure 5

legging was fitted so that the resistance wire lay in contact with the area over the muscles in the back of the leg. The results are shown in figure 6. In (A) the procedure was as before. The subject sat with the feet on the pedals but motionless until the work commenced at the point A. In this case there was no evidence of an initial rise but an immediate drop of temperature. After 4 or 5 minutes the temperature became constant at a lower level and showed some tendency to rise. At B a new fall began, presumably due to sweating. When work ceased at C a sudden temporary rise in temperature was seen, then a final steady rise to the original temperature as with the temperature of the trunk. It seemed

possible that the differences in behavior from the latter were due to the increase in the ventilation of the skin of the leg when pedaling began. To test this the experiment shown in figure 6 (B) was made. Here after sitting at rest for several minutes, pedaling was started at A with no brake load on the wheen, so that very little external work was being done. The immediate drop to a lower level of temperature

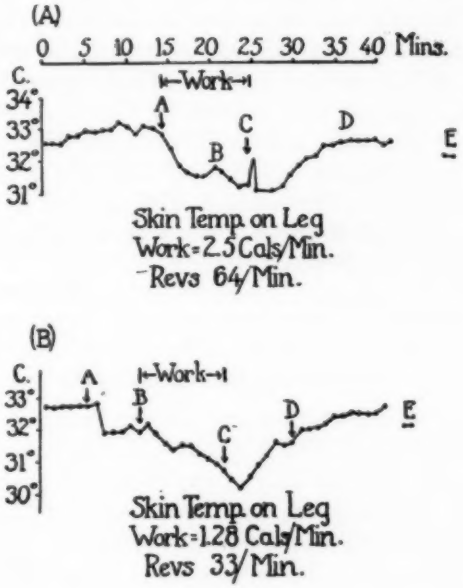

Figure 6

shows how great was the effect of the motion of the air. At B the magnetic brake was switched on, and there was now some indication of an initial rise, followed by the normal fall. At C the load was removed but free pedaling continued until the temperature had reached constancy at D, when the pedaling ceased. There then occurred a new rise of temperature until the original resting level was reached. When the effect of the motion in creating a cooling wind on the legs is taken

into account, the changes in skin temperature on the leg closely parallel those of the trunk.

A great many more experiments would be necessary in order that the onset of sweating might be correlated with other physical factors. It certainly appears from these experiments that the absolute level of the temperature of the skin does not play a dominating part in controlling the onset of sweating in muscular exercise. If it did so, we would expect to find that the maximum temperature reached by the skin would be constant. On the contrary, the maximum temperatures and those at which sweating occurred (the points B on the diagrams) varied from 31°C. to 34.5°C., the latter being under the cloth jacket. There is no indication that in the cooler environment the onset of sweating is delayed. The stimulation of sweating must in the case of exercise be referred to some other factor than sensory stimulus from the skin—possibly to chemical changes in the blood. The correlation with the amount of work done is, however, not at all good.

SUMMARY AND CONCLUSIONS

A technic is described, adaptable for continuous recording, which gives directly the average skin temperature over an extended area such as that of the trunk, with very little disturbance of normal conditions. A considerable length of fine insulated nickel wire is fixed by sewing to the inside of a jacket of light gauze or cloth, so that it is held in close contact with the skin of the subject. Its resistance is balanced in a Wheatstone bridge against a coil of fixed resistance, or against a similarly constructed resistance of nickel wire which is kept at room temperature. Deflection of the galvanometer records in the first case the level of the skin temperature, or in the second case, the excess temperature between skin and room. The use of the method is illustrated by experiments on the skin temperature of a subject doing muscular work on a bicycle ergometer.

1. During muscular exercise there is an initial tendency for the skin temperature of the trunk and leg to rise. This rise may be obscured by the cooling effect of the wind caused by the motion.

2. The rise is quickly halted and followed by a fall of temperature, starting quite definitely at a sharp point, which corresponds to the subjective sensation of sweating. The evidence suggests that this fall is due to the cooling by evaporation of sweat, rather than to the shift of blood flow suggested by Benedict and Parmenter.

3. Upon cessation of exercise, the fall of skin temperature is accelerated, while evaporation continues and the extra heat is no longer brought up to the skin.

4. For some 10 to 15 minutes after the exercise the skin temperature is depressed up to 1½ or 2°C. depending on the severity of the exercise, below the normal level.

5. As the skin dries out and sweating ceases, the original level is gradually approached.

6. The onset of sweating in muscular exercise is not controlled by the level of skin temperature reached, but must be referred to some other factor.

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THE APPLICATION OF THE THEORY OF HEAT FLOW TO THE STUDY OF ENERGY METABOLISM

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FIVE FIGURES

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Since the days of Lavoisier (1777) the animal body has been recognized as a generator of heat. The development of respiration calorimetry has placed the knowledge of this generation of heat by metabolic processes upon a fairly exact quantitative basis. This generated heat eventually becomes heat lost from the body to its environment. Here 'direct calorimetry' has provided quantitative information as to the heat loss and its partition into the various modes of dissipation, namely, radiation and convection, conduction and evaporation of moisture. The transition processes by which heat generated within the body becomes heat lost from the surface have not, however, received much attention, and few attempts have been made to apply quantitative laws in this field. Notable among the attempts were the experiments and conclusions of Lefèvre in his work on 'Animal Heat' ('11). Yet there seems to be possible a considerable advance in clarity of thought, if in nothing else, by the study of this transfer of heat in the light of the laws so well known to apply to purely physical systems. If these laws are held to be of little application to the living animal body on the score that this is something more than a purely physical system yet the question of how much and of what nature this 'something more' may be, cannot be answered until the behavior of the

underlying purely physical system is understood. In that sense physiology must start with physics.

In the application of these laws that follow, a fundamental supposition is that the body is in a state of thermal equilibrium, that is, that heat production is balanced by heat loss so that the temperature of any given part of the system remains constant in time. It is probable that this assumption is never completely justified in the case of the human body. Changes in the heat production, due to muscular activity, ingestion of food and a great number of other factors, cannot be accurately and immediately compensated by corresponding changes in heat loss. The result is the well-known fluctuation of the average temperature of the body as a whole and of the distribution of temperature in its parts. The regulating mechanism possessed by the warm-blooded animals operates to prevent this normal fluctuation of temperature from exceeding limits that are quite narrow, so that over a long period of time heat loss is accurately equal to heat production. Were this assumption of thermal equilibrium not made, the mathematical analysis would become much more complex. Partial differential equations containing time as well as position as a variable would be involved. To a certain extent, the results in cases where thermal equilibrium is not attained may be deduced from the results of the simpler case. It must be remembered that the results here cited are applicable strictly only to the case where the simplifying assumption is justified.

The fundamental law of heat flow is that it does so spontaneously only from a place of higher to one of lower temperature. For any flow to take place there must be a 'gradient of temperature.' This law is, of course, intimately connected with the second law of thermodynamics and it may be taken as established that any apparent contradictions cannot stand upon examination. Further, the flow of heat increases as the gradient of temperature becomes greater. As an illustration of the importance of this law in the case of the human body, we might imagine that we were able to revive miracu-

lously the metabolic processes in a lifeless body that has cooled to the temperature of its surroundings. The heat once more generated by metabolism would be at first totally, later partially, used in raising the body temperature. Since initially there was no gradient of temperature no heat could flow to the surroundings. As, however, the body temperature rose, a temperature gradient of increasing magnitude would be established and an increasing heat loss to the surroundings. Finally, a steady state would be reached when the gradient was sufficient to give a heat flow to the surroundings that exactly balanced the heat generated in the body. There would then be no further rise of body temperature. (A simple calculation shows, however, that it would take at least 15 hours at the normal basal rate for the body temperature to reach to within one-tenth of the final temperature above the surroundings. This is for a body of 80 kg. weight.) This imaginary experiment is closely paralleled by what actually occurs during the waking of the hibernating animal.

The normal body temperature, about 37°C. in humans and a little higher in many mammals and birds, is thus maintained constantly at a higher level than that of the surroundings, which we may suppose to be from 20°C. to 25°C. in normal circumstances. This gradient is sufficient to dissipate all the heat generated by the metabolism. The heat generated in the deep interior of the body is flowing out to the surface, augmented on its way by the heat generated in the more peripheral tissues. This flow demands that there be a gradient of temperature from the interior to the surface of the body as well as a gradient from the surface through the clothing to the surrounding air. Thus, in surroundings at, say 24°C., the average skin temperature over the surface of the clothed body, varying of course considerably in different parts of the body, is from 30° to 33°C., while the internal body temperature is approximately 37°C. A considerable portion of the total drop of temperature from the body to its surroundings thus occurs within the body itself. We may usefully distinguish between the two portions of the total drop of tem-

perature, namely, the internal 'physiological' drop of temperature from the point in the interior of the body where the temperature is a maximum to a given point on the surface, and the external 'physical' drop of temperature from that point on the skin surface to the surrounding air.

This distinction is a natural and a very convenient one. Over the first of these temperature gradients, namely, the physiological internal gradient, man, in common with other warm blooded animals, has a considerable control by regulation of vasomotor activity; over the second, the external gradient, he has only the measure of control that he has learned to exercise by the wearing of clothes and by the conditioning of his environment. It would appear that lack of distinction has led to the occurrence of misleading statements in the literature of energy metabolism. It is reiterated, for instance, by many since Lefèvre made the statement, that the purely physical laws of heat loss have little if any application to the human body as shown by the fact that the heat loss is not found to be correlated with the difference of temperature between the body and its surroundings as these laws would predict (Hoesslin, 1888; Richet, 1889; DuBois, '16). Yet there can be no shadow of doubt that the purely physical laws are applicable to the loss of heat from the surface of the body, the skin, to the air across the external 'physical' gradient. It is in their extension to include the physiological gradient within the body that the physical laws require some modification—a modification which it is the aim of this paper to explain. It has also been argued that the skin temperature is not a reliable guide to the heat loss, since by a modification of the internal gradients within the tissues more heat might be lost without a change in the temperature of the surface (Barr and DuBois, '18). A realization of the purely physical nature of the external gradient of temperature from the skin to the environment shows that this statement is erroneous.

The external gradient. The external gradient will first be considered. The laws that govern the flow of heat from a hot body to its cooler surroundings have long been known. New-

ton summed up his researches on the subject by the well known 'Newton's law of cooling.' It makes no claim to be anything but an empirical law and as such still stands. It states that the rate of cooling, indicative of the rate of loss of heat, is directly proportional to the difference of temperature between the body and its surroundings. The heat loss occurs partly by radiation, partly by convection and conduction, and in the case of the animal body, partly by evaporation of water from the surface of the body. The laws that govern these individual processes are quite widely different. For radiation there is the well-known Stéfan's fourth power law, that the radiation is proportional to the fourth power of the absolute temperature. Its application to radiation from the human body has been shown by the experiments of Aldrich ('28). The laws of convection are complicated and not yet defined to cover all cases, those of conduction are well established. Newton's law is an empirical law applying to the particular combination of these modes of heat loss that are normally found in the case of a body cooling in air. The amount of evaporation occurring is dependent primarily upon the difference of vapor pressure of the moist surface—that of the blood and of the sweat in the sweat ducts—and the vapor pressure of water in the air. In the range of temperature in which we are interested the vapor pressure of water increases with the temperature in a way which, though not linear, is not far from it. The total result of all these factors is that we may apply Newton's law as of the best applicability with simplicity to the loss of heat from the skin. It is also accurately true that in conditions otherwise the same, the loss of heat per unit of time is proportional to the surface area of the hot body. Summarizing this in an equation, if H^1 is the rate of heat loss, say in kilogram calories per hour, T_s the temperature of the skin, A the surface area, and T_ε that of the surroundings:

$$H^1 = C\,(T_s - T_\varepsilon) \times A$$

or dividing throughout by the area, A, and using H for the Calories per square meter per hour—

$$H = C\,(T_s - T_\varepsilon) \qquad (A)$$

The constant 'C' is one that indicates how easily, under given conditions of ventilation, of clothing and of humidity, the heat flows from the skin to the surroundings. It may be called the 'coefficient of heat transfer' from the skin to the environment.

It is perhaps preferable to separate the heat lost by evaporation from that lost by other routes, since this mode may be considered to depend upon a different set of physiological variables from the loss by radiation and convection. We then rewrite equation (A) in the form—

$$H = C\,(T_s - T_r) + H_e \qquad (B)$$

where H_e is the heat lost per unit area per unit time by the evaporation of water.

A simple physical experiment was made to verify that the linear law of heat loss expressed by (A) was valid in the range of temperatures met with in metabolism studies. The bulb of a catathermometer was wound with a coil of resistance wire so that by passing a known current through it the thermometer bulb could be held at a steady state of excess temperature over its surroundings. The coil was covered with a layer of cloth held round the bulb, so that the condition in the experiments to be described might be paralleled. The heat generated, known from the resistance of the coil and the current flowing from it, was in the steady state equal to the heat loss. This was plotted against the excess temperature (fig. 1). The temperatures of the bulb were from 30°C. to 40°C. and the surrounding temperatures from 20°C. to 30°C., the air being still. The reason for the experiment, which must have many times been performed, was to discover how the humidity affected the heat loss by convection and radiation from a hot body in still air.[1] The results show that its effect is negligible for humidities that are encountered in normal conditions. In estimating the measure of heat lost by radiation and convection by the excess temperature above the

[1] Thanks are due to the Eastman Kodak Company of Rochester for the use of a constant temperature and humidity room in these experiments.

surrounding air we therefore use the dry bulb temperature, rather than the wet bulb temperature or some combination of the two such as the 'effective temperature' of Yaglou ('26) and his co-workers. The latter is of significance in the estimation of heat loss by evaporation as well as by other routes, while here we deal with the evaporation loss separately.

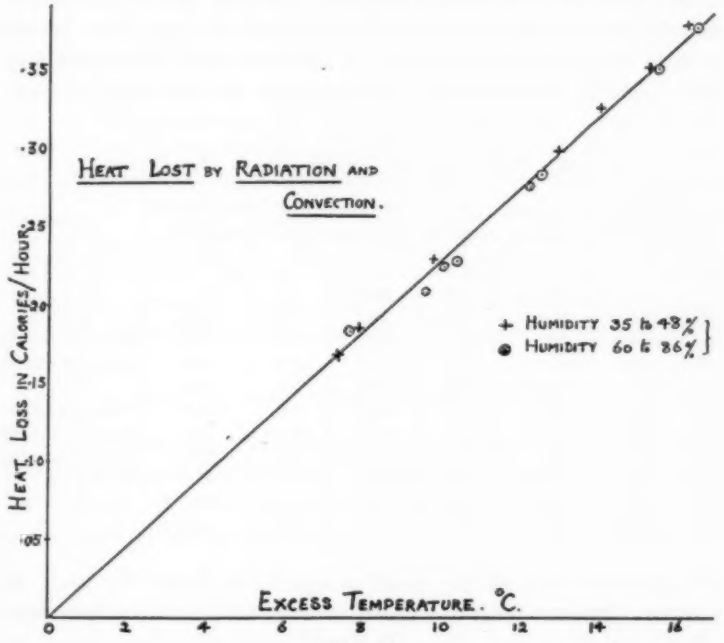

Figure 1

An estimate of the order of magnitude of the coefficient C in normal circumstance for the human body may be easily made. For adults H is about 30 kg.-Calories per square meter per hour. Assuming an average skin temperature under the clothing as 32°C. in an environment of 24°C., the excess temperature is of the order of 8°C. Throwing these quantities into the fundamental units of small calories, square centimeters, and seconds, we find by substitution in equation (A)—

$$C = 1.05 \times 10^{-4} \text{ Cals./sq.cm./sec./°C.}$$

The exact value of C, of course, depends upon the clothing worn. By estimating the surface area of the radiating bulb in the experiment described, the results of which are given in figure 1, a value of the same order results for the coefficient of heat transfer into still air.

Returning to consider equation B, it is a fortunate circumstance that in a variety of conditions and with a diversity of subjects the heat loss by evaporation, H_e, has been found to be a very constant fraction of the total heat loss, provided that there is no sweating. So constant is this fraction that

TABLE 1

W. Leet. Weight, 23.6 kg. Height, 128 cm. Age, 7 years

DATE	ROOM TEMPERATURE	SKIN TEMPERATURE	EXCESS TEMPERATURE	RECTAL TEMPERATURE	CIRCULATION INDEX	METABOLISM CALS./SQ.M./HR.	REMARKS
1932							
12–21	25.5	33.5	8.0	36.7	2.5		
12–22	27.5	35.1	7.6	36.7	4.75	42.7	
12–23	26.65	36.1	9.45	37.2	(8.6)	45.2	Skin temperature
12–24	28.1	36.0	7.9	37.6	4.95	44.7	probably in error
12–25	27.2	35.85	8.65	38.2	3.7	51.9	
12–26	26.4	37.5	11.1	39.0	7.4	54.0	Rash appeared
12–27	27.2	37.7	10.5	38.4	15.0	45.2	Rash at maximum
12–28	27.5	36.9	9.4	38.6	5.5	54.1	Rash decreasing
12–29	26.6	34.65	8.05	38.2	2.3	53.1	Rash gone
12–30	27.0	35.9	8.9	38.7	3.2	45.5	
12–31	26.67	34.3	7.6	37.2	2.6	44.7	

the measurement of the total evaporation from the body in a given time has been used as a measure of the total heat loss (Root and Benedict, '26). It would seem that there must be considerably less error in using the excess temperature, and thus the heat loss by radiation and convection, as an index of the total metabolism, since we deal then with the 75 per cent of the total rather than with the 25 per cent lost by evaporation. The measurement of the average skin temperature over large areas of the body, as has been done by the author using a new technic described elsewhere (Burton, '34), gives a means of estimating very fairly the total heat loss from the body. As an example, table 1 is given, showing

how the excess temperature of the trunk of a subject lying on a bed in still air varied in the course of a fever[2] induced by administrations of 'Nirvinol.' At the height of the fever there was a 50 per cent increase in the excess temperature and therefore in the heat loss by radiation and convection. There was no significant change in the loss by evaporation in this period, sweating not occurring until after the fever had subsided. The metabolism was simultaneously determined by the Tissot-Haldane method.

The heat loss by evaporation is normally about 25 per cent of the total loss. Of this evaporation loss, about 36 per cent is lost from the lungs and the remaining 64 per cent from the skin. The normal loss is thus 25×0.64, or 16 per cent of the total heat loss from the skin, while radiation and convection account for the remaining 75 per cent. We may then assume that the heat lost from an area of skin by evaporation is normally $16/75 = 0.21$ of the heat lost from that area by radiation and convection. Equation B may then be written as:

$$H = C\,(T_s - T_e)\,(1 + 0.21) = 1.21\,C\,(T_s - T_e) \qquad (C)$$

If we measure the evaporation H_e from the skin at the same time as we measure the skin temperature, and write H_{e_0} for the normal value of H_e, which would give just 21 per cent of the total loss, we have—

$$H = C\,(T_s - T_e)\,(1 + 0.21\,H_e/H_{e_0}) \qquad (D)$$

The application of this modification of the equation will be seen later.

The internal gradient. It was pointed out that the flow of heat from the interior of the body to the surface necessitated the existence of the internal 'physiological' gradient of temperature. This flow of heat takes place by the process of pure conduction across the tissues and by the transport of heat by the blood flow from hotter to cooler parts.

[2] These data were compiled with the cooperation of Mr. Rockwell at the Strong Memorial Hospital, Rochester, New York.

The laws of conduction of heat through a plane slab of material of infinitesimal thickness, whose faces are at different temperatures, are very simple. They are that the rate of flow of heat across the slab is proportional, a) to the cross-sectional area, A; b) to the difference of temperature of the two faces ΔT; c) inversely to the thickness of the slab, ΔX; or in an equation—

$$H' = K.A.\frac{\Delta T}{\Delta X}$$

$\frac{\Delta T}{\Delta X}$ is the thermal gradient, G, in degrees centigrade per centimeter. Here K is a constant for the material, the 'specific thermal conductivity.' Dividing by the area A, we have for the heat flow per unit area per unit time—

$$H = K.G \qquad (E)$$

The application of these laws to a case where there are finite thicknesses of material, not necessarily in plane slabs, and finite differences of temperature, involves the use of calculus and integration. The process is one very familiar to students of theory of heat, and is illustrated here simply to show its applicability to the flow of heat in living tissues.

Consider two proximate surfaces between which the heat is flowing, at the first of which the temperature is T_1, the area A_1 and at which the gradient of temperature is G_1. At the second surface the temperature is T_2, the area A_2 (not equal to A_1 unless the heat is flowing in parallel lines across 'plane slabs') and the gradient is G_2. Then the heat flowing in unit time into the section of material across the first face is—

$$H_1 = K A_1 G_1$$

or in the language of the calculus—

$$H_1 = -K.A\frac{dT}{dX}$$

The heat that flows out of the section is—

$$H_2 = K A_2 G_2, \text{ or } H_2 = -(K A \frac{dT}{dX} + \frac{\delta}{\delta X}[K A \frac{dT}{dX}] dX)$$

These two must be equal unless there is retention of heat within the layer, which would therefore rise in temperature. In dealing with the 'steady state' only we exclude this term. If, however, heat is being generated within the layer, or amount, say 'h' Calories per second per cubic centimeter, the heat leaving the layer will be in excess of that entering it by the amount of the heat generated in the layer. The equation results:

$$H_2 - H_1 = -\frac{\delta}{\delta X}\left[K\,A\,\frac{dT}{dX}\right] dX = A.\,dX.\,h.$$

or

$$\frac{\delta}{\delta X}\left[K.\,A.\,\frac{dT}{dX}\right] = -A.\,h. \qquad (F)$$

The integration of the equation when applied to bodies of various shapes gives the distribution of temperature throughout the body, i.e., the form of the gradients in the different parts.

The same mathematical analysis is adequate to describe the actual flow of heat in living tissues, where a major part of the flow may be through transport of blood. Such transport across the boundaries of the section may be resolved into two components, a flow in the direction of the heat flow at right angles to the faces, and one perpendicular to this. The former may be taken account of by considering that it effectively increases 'K,' the conductivity, that appears in the equation. The lateral flow which leaves heat in the section is mathematically equivalent to an increase in the heat generated in the section, the 'h' of the equation (F). The general results of the integration of (F) in special cases have therefore some application to the temperature distribution in the body.

The details of the integration in the case of bodies that have simple geometrical shapes, such as the cylinder and sphere, are to be found in almost any advanced textbook on heat. No useful purpose would be served by their repetition here. Some of the general principles that emerge may be cited as of interest in their application to the thermal gradients in the living body.

1. In a uniform medium in which heat flows in parallel lines, as through a slab where the temperature is uniform over a cross-sectional area, the drop of temperature is linear if no heat is generated in the medium itself. The gradient is therefore constant. For the same amount of heat flow per unit area of cross section, this gradient is greater the poorer the conductivity of the medium. The gradient thus tends to be

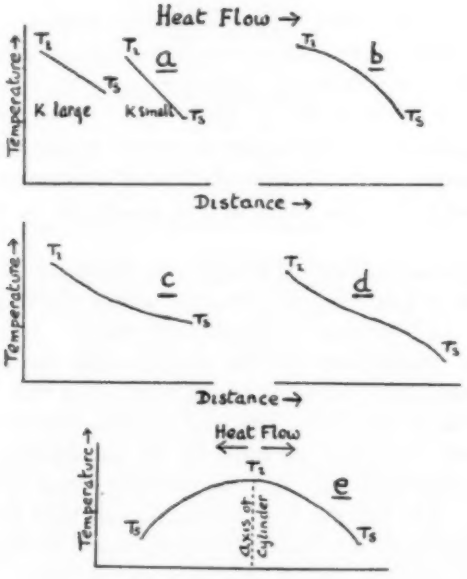

Figure 2

steeper in regions of small vascularity or of good thermal insulation, such as layers of adipose tissue. Figure 2 a illustrates this simple case.

2. If in addition to this 'passive' transfer of heat across the slab there is heat generated uniformly within it, the curve of temperature with distance becomes parabolic instead of linear, the gradient becoming steeper as we progress in the direction of heat flow (fig. 2 b).

3. When the heat flows, not in parallel, but in diverging lines, as when it is flowing from the center to the surface of a cylinder or sphere, the gradient is not uniform even if we have no heat generated. Due to the increasing cross-sectional area through which the heat flows the gradient becomes less as we pass outward to the surface. The case of a cylinder introduces a logarithmic term, that of a sphere, a term involving the reciprocal of the radial distance, but in each case the curve is of the form shown in figure 2 c.

4. When to the last case we add that heat is uniformly generated within the medium, a parabolic term is added as it was in b and the result is a complex curve as in figure 2 d. At the greater radial distances this latter term, the parabolic one, will predominate and the curve will tend to become convex upward, i.e., toward the direction of higher temperatures. In certain cases, as of a cylinder or sphere in which heat is generated uniformly throughout its volume, the equations become simpler, reducing a purely parabolic distribution of temperature (fig. 2 e).

We learn then that even in the case of a body of uniform thermal properties the distribution of temperature is not in general linear. The flow of blood and the generation of heat within the tissues tend to produce the parabolic type of temperature distribution, while the diverging nature of the flow tends to give the opposite curvature, particularly evident in regions of thermal inactivity. Temperatures at different depths in the human forearm and thigh have been measured by Bazett and McGlone ('27) and at different depths in the rectum and vagina by Benedict and Slack ('11). Their curves show that, as predicted, the distribution of temperature is in general parabolic. Had the analysis not been made, it might have been erroneously concluded that the increase of gradient near the periphery necessarily indicated a decreased conductivity in these regions. This may be the cause, but even in a perfectly uniform mass of tissue, the changing gradient would be evident if heat were generated in the tissues through which the heat flowed.

The equations give the value of the temperature T at a distance X, in terms of the quantities K, h, defined above, the temperature T_i at some designated internal point, and the heat flow H per unit area across some designated surface. If for the distance 'X' the linear dimension of the body, as 'R_s' the radius of the cylinder or sphere, be substituted, the temperature T_s of the surface of the body results. 'H' is then the heat flow per unit area at the surface. The results vary widely, of course, according to the details of the problem dealt with, but upon examination a characteristic common to all of them appears. It is that they can all be given the form—

$$T_i - T_s = \frac{H R_s}{K} \cdot f \qquad (G)$$

In this equation, f is a function whose form and magnitude depend upon the particular geometry of the problem chosen—the shape of the body, the position of the point where the temperature T_i is stated, and so on. The importance of this result is that we may write—

$$H = C' (T_i - H_s) \qquad (H)$$

where C′ is a coefficient of heat transfer (as was C in equation (A)) for the transfer of heat from the interior of the body to a point on the surface. In addition, we know that in bodies which are similar in geometry and specific thermal properties and which differ only in the size factor, this coefficient C′ varies inversely as the linear dimensions.

$$C' = \frac{A}{R_s} \qquad (J)$$

Though this has been deduced from consideration of a number of special cases, it can be shown to be a general theorum applying whatever the details of the problem chosen may be. Equation (J) will be used later in this paper.

An estimate of the order of magnitude of the thermal conductivity of tissue may easily be made. The measurements on the gradient of temperature in human subjects shows that there is a rise of temperature of about 5°C. (from 32° to 37°C.) to a depth of some 2.5 cm. The average gradient is

then about 2°C. per centimeter. For H, the heat reaching from the surface per square centimeter we may assume that the heat loss is the same as the average for the whole body, namely, about 40 kg.-Calories per square meter. Using equation (E) and reducing the quantities to the appropriate c.g.s. units, we find—

$$K = 0.0004 \text{ Cals./sq.cm./sec./°C./cm.}$$

Comparing this with the values of the coefficient of conductivity K for common substances, we find that K for glass is 0.002, for water 0.0015, for wood 0.0004 and for flannel 0.0002. The tissues of the human body, in spite of circulation of the blood, are surprisingly effective as heat insulation. Lefèvre obtained values by direct measurement of the same order of magnitude for this quantity.

'The thermal circulation index.' We have equation (A), in which form the modified equation (B) can be written also:

$$H = C\,(T_s - T_e)$$

and equation (H)—

$$H = C'\,(T_i - T_s)$$

The heat coming up to a particular area of skin must equal the heat leaving that area to the surroundings. The two quantities H in these equations are therefore identical, and we have two simultaneous equations, from which we may eliminate one of the variables. The simple process of division eliminates H and we have—

$$\frac{C'}{C} = \frac{T_s - T_e}{T_i - T_s} = r \qquad (K)$$

The thermal conductivity of the underlying tissues may therefore be compared with that from the skin to the surrounding air by the calculation of this ratio. Under controlled conditions of humidity and ventilation the coefficient C may be made constant. Measurement of the skin temperature under these controlled conditions therefore enables us to follow changes in the coefficient C′ which indicates the ease with which heat is transferred from the interior of the body to the area of skin chosen.

It has been recognized that the temperature of the skin depends on a great variety of factors, namely, the environmental conditions, the internal temperature of the body, the clothing, as well as upon the physiological conditions of the circulation. It is impossible to keep constant all these factors save the particular one that it is desired to study and thus the great quantity of data on skin temperatures to be found in the literature is hard to interpret. G. N. Stewart in one of his last papers ('30) remarked that there is no great difficulty in measuring the temperature of the skin, but that it was difficult to know what to do with the data once obtained. By the use of the ratio above, however, we eliminate at once the primary 'per se' influence of several factors, namely, of the environmental temperature, of the internal temperature and of the level of the metabolism. If the remaining factors that govern the coefficient 'C,' namely, the humidity, the ventilation and the clothing be controlled, we have left in this ratio a quantity that is an index of the physiological state of the tissues. Changes in the index will be chiefly due to changes in the peripheral circulation, while water and fat content will also play a part in determining its normal value. It may therefore be called a 'thermal circulation index.' An increase in its value indicates an increased conductivity for heat from interior to the surface of the body, which would be brought about by an increased peripheral circulation.

$$\text{Thermal circulation index, } r = \frac{\text{External drop of temperature}}{\text{Internal drop of temperature}}$$

The secondary influence of the other factors of environment and so on are of course not eliminated by its use, but their influence upon the physiological state of the tissues is shown by the change they induce in the index 'r.' In a warm atmosphere, for example, we should expect to find that the circulation index was increased. For a purely physical system, in which there could be no change in the coefficient C′, though the surface temperature would of course rise in a warmer environment, the rise would be such that the value of the index would prove to be unchanged.

The numerical value of the index will be greatly different when calculated for different parts of the body, since it indicates the relative ease with which heat reaches the particular area of surface chosen, from the interior of the body where the hottest temperatures prevail. Its value depends upon the length of the path traversed by heat to that part, upon blood supply of the part, and upon the amount of superficial fat at the point. Table 2 gives temperatures taken by thermocouple measurements upon a subject after lying nude in an environ-

TABLE 2

Skin temperatures on subject (G.McC.) after lying nude for 20 minutes in room at 22.8°C. Rectal temperature = 37.25°C.

POSITION	SKIN TEMPERATURE °C.	EXCESS TEMPERATURE °C.	INTERNAL DROP °C.	CIRCULATION INDEX
Forehead	33.40	10.60	3.85	2.75
Clavicle	33.60	10.80	3.65	2.96
Over breast	32.75	9.95	4.50	2.21
1 inch over umbilicus	34.20	11.40	3.05	3.75
Over apex of heart	33.30	10.50	3.95	2.67
Lumbar region	33.30	10.50	3.95	2.67
Arm, biceps	32.85	10.05	4.40	2.28
Palm of hand	32.85	10.05	4.40	2.28
Knee cap	32.35	9.55	4.90	1.95
Calf of leg	32.20	9.40	5.05	1.86
Sole of foot	30.20	7.40	7.05	1.05
Big toe	30.95	8.15	6.30	1.29

ment of constant temperature. In the calculation of the circulation index the rectal temperature is used as the 'internal temperature' for lack of a better. The values of the index indicate, for instance, that heat from the interior reaches the surface of the abdomen three times more easily than it does the sole of the foot.

In order to make studies of circulatory changes a particular area of skin, such as that of the trunk is chosen, and the changes in the index for that area observed. If changes in the evaporation are suspected, the index may be corrected for these if the evaporation from the area is measured and com-

pared with the normal evaporation. For, using the modified equation (D), we would obtain—

$$r = \frac{C'}{C} = \frac{T_s - T_e}{T_i - T_s}\left(1 + 0.21\,\frac{H_s}{H_{so}}\right)$$

The uncorrected values of the index are then to be modified by multiplication by the factor $(1 + 0.21\,\frac{H_s}{H_{so}})$. An increase in the evaporation to double the normal amount by sweating would thus produce an error in the apparent circulation index of about 20 per cent only. In the preliminary work reported here, these corrections have not been made.

Variability of the index. Preliminary studies of the skin temperatures over the trunk and over the leg of a normal subject indicate that the normal variability of the circulation index, in quite a wide range of environmental temperatures, is of the order of 20 per cent for the trunk, and 30 per cent for the leg.

In abnormal conditions of the circulation, however, as in fever, there are very much greater changes than this. The seventh column of table 1, for instance, indicates how in the height of the fever and erythema of the skin the index had increased some four- or fivefold. In a case of severe edema (hives) without fever, the index showed a two- or threefold increase, presumably due to the increased thermal conductivity of tissues containing water.

It is well known that under local or general anaesthesia there is practically complete vasodilatation of the peripheral vessels which is accompanied by a rise of temperature of the skin, particularly of the extremities. Morton and Scott ('30), among others, have used this as an indication of sympathetic vasoconstrictor activity. Some of their results are given in table 3.

TABLE 3

	ROOM TEMPERATURE °C.	TOE TEMPERATURE °C.	CIRCULATION INDEX	FACTOR OF CHANGE
Initial	25.4	27.0	0.16	
After $NO_2 + O_2$	25.4	28.5	0.36	2.2
After ether	25.4	33.0	1.90	12.0

Rectal temperature is assumed to be 37.0°C.

Under complete anaesthesia the index for the toe changed by a factor of twelve times. This large change is not as surprising as at first sight when Poiseuille's law of the flow of liquids through a tube of small diameter is referred to. It states that increase of diameter of the capillaries to twice their former size would result in a sixteenfold increase in the blood flow through them and through the superficial skin. (Flow varies as fourth power of diameter.) Changes of diameter of capillaries of much greater magnitude than this are known to take place. The work of Stewart, of Pickering, of Grant, Bland and Camp ('32) and others, on the vascular reactions of the rabbit's ear, is of interest in this connection. Their results show variations in the circulation index of similar magnitude. Incidentally the usefulness of the index is here illustrated. The ear is first immersed in a water bath at say 15°C. and its surface temperature noted by the use of thermocouples. The temperature of the water bath is then changed to say 27°C. and the temperature of the surface of the ear is found to rise. Whether this rise is simply that which would naturally follow in a purely physical system, or whether it involves a vascular response may be discovered by calculation of the index in the two cases. An increase in the index means that there has been vasodilatation; a decrease, vasoconstriction.

The dependence of skin temperature upon environment and body temperatures. Equation (K) may be rearranged to express the dependence of skin temperature upon the environmental temperature T_ε and upon the internal temperature T_i. It yields

$$T_s = \left(\frac{1}{1+r}\right) T_\varepsilon + \frac{r}{1+r} T_i \qquad (L)$$

Talbot ('31), in his studies on the skin temperatures of children, remarked upon the fact that the temperature of the extremities was very sensitive to changes in environmental temperature but relatively insensitive to changes in body temperature, while of the temperature of the chest the reverse was true. That this follows from the equation above is shown

by table 4, which is constructed directly from that equation assuming convenient values for room and for internal temperatures. The extremities are at temperatures of about 28°C. while the chest may be taken to be at about 33°C. in such circumstances.

Vincent (1890) from his studies derived a correction factor which should be subtracted from the skin temperature to reduce all values to a standard room temperature, which was the 20°C. used in the above table. The correction he gives as 0.3°C. per degree rise of room temperature, the equation being—

$$T_s = 26.5 + 0.3\,T_e.$$

This is the value predicted by the table for skin temperatures at about 31°C.—a good average value for the skin temperature over the body. Reichenbach and Hegmann ('07) give a formula for the dependence of the skin temperature (H) upon air temperature (L) and upon internal temperature (B).

$$H = \frac{B}{K} + \frac{K-1}{K} L$$

where K is a numerical constant. In the symbols used in our equation this is—

$$T_s = \frac{K-1}{K} \cdot T_e + \frac{1}{K} T_i$$

The equation is identical with that derived from pure physical principles if K is put equal to $(1 + \frac{1}{r})$. These authors give the physical derivation in much the same way as it has been developed here, but do not note that the dependence of skin temperature upon room temperature itself depends upon, and can be predicted from (as in table 4) the level of the skin temperature at a particular room temperature.

TABLE 4

Room 20°C., rectal 37°C.

SKIN TEMPERATURE	INDEX C'/C	CHANGE FOR 1° ROOM	CHANGE FOR 1° RECTAL
°		°C.	°C.
35	7.5	0.18	0.88
33	3.3	0.23	0.77
30	1.4	0.42	0.58
28	0.90	0.47	0.53
25	0.42	0.70	0.30
23	0.21	0.83	0.17

It is well known also, that there are greater fluctuations due to circulatory changes, in the temperatures of the extremities than of the warmer parts of the skin. This also may be deduced from the equation (L) by tracing the dependence of skin temperature T_s upon the index r, the other factors remaining constant. Table 5 gives the results. A

TABLE 5

Per cent change in index for 1°C. in skin temperature $= \left(\frac{1}{T_s - T_e} + \frac{1}{T_i - T_s}\right) \times 100$

SKIN TEMPERATURE °	INDEX C'/C	PER CENT AGE CHANGE IN INDEX FOR 1° CHANGE IN SKIN TEMPERATURE	
35	7.5	57	
33	3.25	33	Room 20°C.
30	1.43	24	Rectal 37°C.
28.5	1.00	12	
27	0.70	22	
25	0.42	28	
23	0.21	40	

change of 1°C. in skin temperature of the chest indicates a far greater change in circulation than an equal change of temperature of the extremities. The maximum sensitivity of skin temperature to circulatory changes occurs when the skin temperature is just halfway between the environmental and internal temperatures. The temperature of the extremities happens to lie nearer this maximum condition than does that of the chest. It is not justifiable, therefore, to conclude from

the greater variation in temperature of the extremities that circulation changes are greater here, although this may be true. Reliable simultaneous measurements made upon skin temperatures of different parts of the body under different environmental temperatures would be necessary to decide whether vasometer adjustments, as in the heat regulation of the body, are general or localized in particular parts of the body. The results of preliminary experiments upon the skin temperature of the chest and of the leg in different environmental temperatures have so far indicated that the index changes very little, if at all appreciably, in the range of temperature from 20 to 30°C. If this is confirmed it means that the part played by dilatation of the peripheral vessels in the

TABLE 6

J. Stewart, weight, 29.2 kg. Height, 131 cm. Age, 8 years

DATE	ROOM TEMPERATURE	SKIN TEMPERATURE	EXCESS TEMPERATURE	RECTAL TEMPERATURE	CIRCULATION INDEX	METABOLISM CALS./SQ.M.	REMARKS
3–5–33	22.28	33.40	11.20	37.5	2.7	43.8	
3–6–33	23.15	32.15	9.0	37.0	1.85	45.1	
3–7–33	27.46	33.96	6.50	37.4	1.9	45.6	No skin
3–8–33	24.52	33.11	8.59	37.2	2.1	48.4	reaction
3–9–33	19.38	31.25	11.87	37.5	1.9	42.4	

regulation of heat loss in man at any rate in the normal range of environmental temperatures, has been greatly overestimated. As an example, table 6 is given. In this case the subject did not react to the drug (Nirvinol) and no fever or skin reaction was evident. The constancy of the index in widely different room temperatures is remarkable when compared to the variation shown in table 1, where fever was induced. Lefèvre (loc. cit., p. 399) found that the conductivity of the superficial layers of the skin was twice as great when the subject was immersed in a bath at 30°C. as when in one at 5°C.—these, however, are extreme limits of temperature. The normal level of the average skin temperature being about 32°C. in surroundings of say 24°C., the excess temperature is of the order of 8°C. Even if the conductivity

were increased to an infinite value the skin temperature could not rise to a value above the body temperature, 37°C. The excess temperature would then be increased to 13°C.—an increase of only some 50 per cent. Vasodilatation is, therefore, quite inadequate to deal with the increases of 200 to 1000 per cent in heat loss that are necessary in exercise. Here sweating must be, and is, called upon to get rid of the excess heat by evaporation.

Metabolism and environmental temperature. Returning to the two fundamental simultaneous equations (A) and (H),

$$H = C\,(T_s - T_e)$$
$$H = C'\,(T_i - T_s)$$

we may eliminate the skin temperature T_s instead of the metabolic heat loss per unit area, H. The result of this elimination is the equation:

$$H = C\,\frac{(T_i - T_e)}{1 + \frac{C'}{C}} = C\,\frac{(T_i - T_e)}{1 + \frac{1}{r}} \qquad (M)$$

In this equation 'C' and 'r' refer to the external coefficient of heat transfer and the circulation index, respectively, for the particular area of surface chosen, while H is the heat loss, say Calories per square meter per hour, from this area. A relation of the same form as (M) will hold between similar quantities which are average values for the whole surface of the body. H will then be the average heat loss in Calories per square meter per hour, the quantity (equal in the long period of time to the heat production) which is measured in studies of metabolism.

Equation (M) emphasizes the fact that if the metabolism of the body changes there must be a compensating change in the quantities on the right hand side of the equation. Unless the environment and clothing are changed, an increase in the metabolism must result in a change in the internal temperature T_i, if there be no compensating change in the index 'r.' Changes in circulation must accompany changes in the level of metabolism if the body temperature is to re-

main within the limits suitable to life. The increase in the index found in fever is an indication of this compensating change of circulation. Similarly, when the environment is changed, 'Tε' changing, either the metabolism must change so that the internal temperature may remain constant, the 'chemical regulation' of Rubner, or the index r must change by a change of circulation, sweating or peripheral dilatation or constriction. The latter is the 'physical regulation.' The equation therefore expresses in a concise form these considerations.

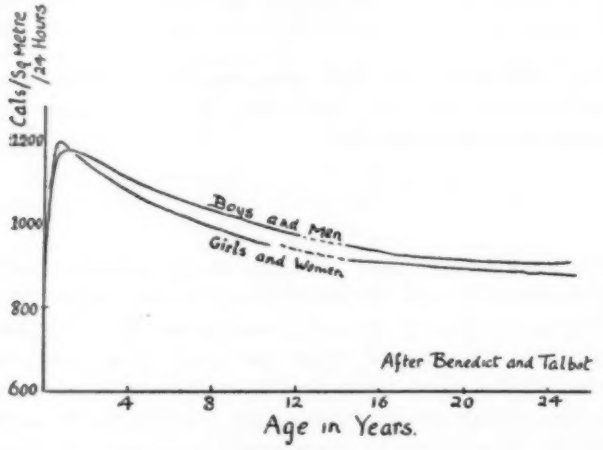

Figure 3

Metabolism and size. When equation M is applied to the consideration of the changes in metabolism per unit area during growth, interesting conclusions are indicated.

In the well-known human 'age curve' (fig. 3) the metabolism per unit area is plotted for different ages. The accepted curve is the result of averages of a great number of determinations of basal metabolism upon a number of subjects by Benedict, Murlin, DuBois, Boothby and Sandiford and others. For the average curve that results, we may justifiably assume that the temperature of the environment, Tε, was the

same for all ages, although this factor has not been controlled in experiments upon normal basal metabolism as well as would be desirable and there must have been considerable variation in the individual cases from which the average curve was derived. The coefficient C we may assume also to be sensibly constant. If the environment were colder, making the difference $(T_i - T\varepsilon)$ greater, the tendency would be to increase the clothing of the subject for him to be 'comfortable' and thus decrease C for this particular case. The tendency is then for variations in the two factors $T\varepsilon$ and C to compensate so that on the average the result is constancy. There remains the possibility that children are habitually clothed more warmly, or perhaps, less warmly, then adults so that C is not constant at different ages. This cannot be denied, but it does not seem likely in view of the fact that the scatter of individual points is not greater than it is. The regulation mechanism, by which the metabolism is rendered relatively constant in spite of changes in the amount of clothing also tends to eliminate the changes of this factor. In the case of infants the habitually greater clothing may play some part. What follows is based on the assumption that the same type of curve would have been obtained if the metabolism had been measured on all subjects lying unclothed in rooms of a standard temperature. The remaining factor of the numerator of (M), the internal temperature, is on the average the same—about 37°C. for all ages. For young children it is slightly higher, 37.5°C., but the difference is insignificant in view of the large changes in the metabolism curve. These changes may then be referred to a change in the normal value of the thermal circulation index 'r' that occurs in the denominator of the fraction in (M).

As the body increases in size, the absolute amount of tissue through which the heat must be transferred to reach the surface increases. It was shown that, if the thermal nature of the tissues and circulation remain the same, for bodies that are thermally and geometrically 'similar' this size factor changes the value of the coefficient C′ by the equation (J)

$C' = \frac{A}{R_s}$. Thus the circulation index, $\frac{C'}{C}$ for such 'similar' bodies would vary inversely as the linear dimensions of the body. Thus we may rewrite equation (M) as—

$$H = \frac{B}{1 + aL} \qquad (N)$$

'L' being a linear dimension of the body. 'B' and 'a' are constants. The metabolism per unit area of the smaller body would (for such 'similar' bodies) be therefore greater. In simple terms, the smaller body has less insulation in absolute amount. There is then less drop of temperature from interior to surface, and, the internal temperature being the same, the average skin temperature is higher, so that the heat loss and the metabolism per unit area are therefore greater. The experimental observation is that after the maximum at the age of 1½ to 2 years, the metabolism per square meter does actually decrease with increasing size.

For an estimate of how great a decrease the size factor would produce on the heat loss per unit area, we may take the cube root of the weight as proportional to the linear dimensions of the body, writing the equation—

$$H = \frac{B}{1 + A' W^{\frac{1}{3}}} \qquad (O)$$

The value of the constant A' is known if we know the value of the average circulation index at any given weight. If, for instance, we assume a thermal circulation index of 2 for 70 kg. man, which means that his average skin temperature is about 31°C. in an environment at 20°C., we have, since $A' W^{\frac{1}{3}} = \frac{1}{r}$

$$A' = \frac{1}{2 \times \sqrt[3]{70}} = 0.121$$

and

$$H = \frac{B}{1 + 0.121 W^{\frac{1}{3}}}$$

Substituting the measured value for H, 925 Cals./sq.meter/24 hours, for the weight 70 kg., we obtain the value of B.

$$B = 1388, \text{ and } H = \frac{1388}{1 + 0.121 W^{\frac{1}{3}}}$$

Thus it is possible, knowing the level of the metabolism at a given weight and the average circulation index, which involves a knowledge of the average skin temperature over the body in surroundings of a given temperature, to predict what the metabolism per unit area would be in bodies that were 'similar' but of other weights.

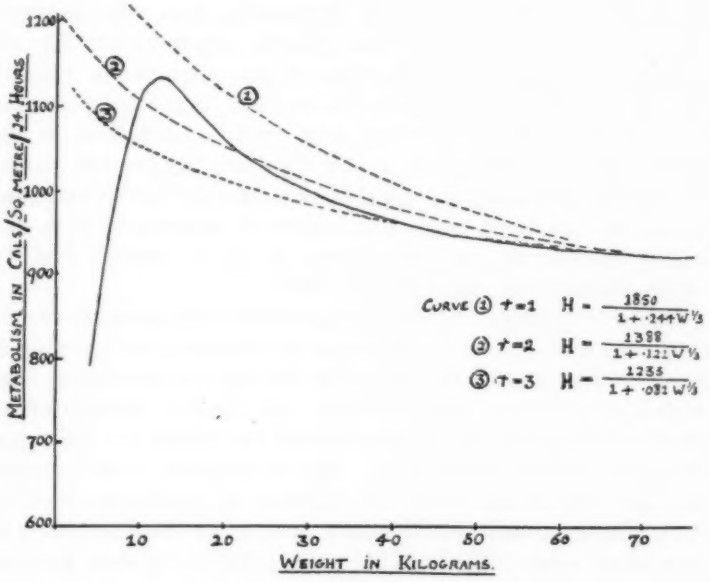

Figure 4

In figure 4 is plotted in the continuous line the experimentally determined curve of metabolism per square meter against weight, after Benedict and Talbot ('21). On the same chart the dotted lines represent the result of the size factor, calculated as indicated above, assuming the different values 1, 2 and 3 for the circulation index 'r' of the 70 kg. man. It is seen that for an index between 2 and 3, the fall of the metabolism per unit area, after the maximum that occurs at about 15 kg., is of about the same order as would be produced

by the size factor alone. The scatter of the points from which the experimental curve was deduced are so great near the maximum that the true curve might well coincide with the calculated curves from that point on. It is therefore possible to attribute the progressive decline in the metabolism per unit area after the maximum that occurs at the age of 1 or 2 years to the increase in the absolute amount of thermal insulation of the body with increasing size, the thermal nature of that insulation (its specific conductivity K) remaining constant. Confirmation of this view of the role of thermal insulation is found in the fact that in the age curve for men the metabolism per unit area becomes constant, or attains a much smaller slope, at just the age when growth ceases. In women, however, the level is consistently lower and continues to fall until later life. This is consistent with the known greater thermal protection of fat in women and its continued increase until middle life.

Below this critical weight that gives the maximum, however, there is a wide departure between the observed and predicted curves. In spite of the fact that its size is increasing, and with it, if it were a 'similar' body, the absolute amount of its thermal insulation, the metabolism of the infant per unit area increases rapidly from birth. The conclusion is inescapable that for the infant either the amount of insulation relative to its linear dimensions is much greater or the insulation is of a much more effective kind. An estimate of how great a change in the thermal properties of the insulating tissues would be necessary, may be made on the assumption that the relative amount is the same, varying as the cube root of the weight, as was assumed for the rest of the curve. The whole change is then attributed to the change in the coefficient of conductivity 'K' which occurs, by equation (G), in the coefficient C′ as $\frac{R_s}{K}$. Equation (O) is then written—

$$H = \frac{B}{1 + \frac{bW^{\frac{1}{3}}}{K}}$$

Where b is a constant. If we know that the circulation index is r at the adult weight (70 kg.) at which the metabolism is H_o we have also

$$H_o = \frac{B}{1+\frac{1}{r}}$$

Solving we obtain

$$K \propto \frac{W^{\frac{1}{3}}}{\left(1+\frac{1}{r}\right)\frac{H_o}{H}-1}$$

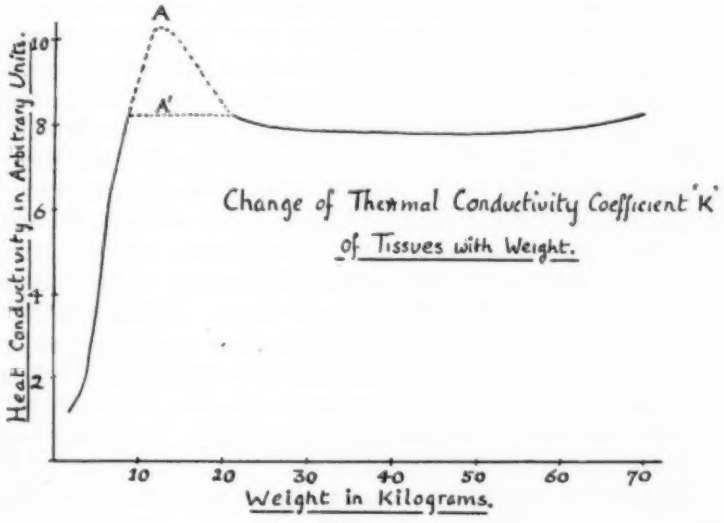

Figure 5

The changes in 'K' thus deduced from the experimental curve of metabolism are shown in figure 5, where the index r for the adult is chosen as $r = 2$. It is seen that the insulation of the infant must be some five or six times as effective as that of the adult, and that there is a progressive change in conductivity until the age of 1½ to 2 years, after which the conductivity is sensibly constant. The peak at A cannot be taken to be significant, since it is the peculiarity of the equation that in this region a change of only 4 per cent in the

metabolism per unit area, H, would bring the curve for K down to A′. It is not claimed that the experimental curve of figure 4 has a certainty of anything like this 4 per cent, especially in the region referred to. In the region of lower weights, however, a much greater change in the metabolism curve would be necessary to change the curve of figure 5 very much. Very little change in the general conclusions is made if instead of $r = 2$, $r = 1$ or $r = 3$ is taken. It is difficult to assign a good average value for the skin temperature over the whole body under the 'normal' clothing in 'normal' conditions, but in a room temperature of 20°C. that average temperature, from the data in the literature, lies probably between the limits of 27.5°C. and 33°C., which would correspond to values of the circulation index 'r' of 1 and 3, respectively.

For this progressive increase in the thermal conductivity of the tissues in the early years of life, two explanations may be suggested. It may be that there is a decrease in fat and an increase in water content of the growing infant. Since water has a thermal conductivity that is three or four times the average thermal conductivity of tissue, and fat considerably less conductivity, this would produce a progressive increase in the conductivity. This influence of fat in controlling heat loss and thus heat production in atrophic and in normal infants has been discussed by Murlin ('23). A second contributing factor, which may be of major importance, would be a progressive increase in the peripheral and general circulation of the growing infant.

It must not be forgotten that, as has been mentioned, the fact that infants are habitually clothed more completely than in later life might vitiate these conclusions to some extent. It is difficult to believe, however, that clothing could change the external coefficient C, and therefore the index $\frac{C'}{C}$ by a factor as great as six times. An experiment upon the skin temperature on the chest of a subject, both nude and fully clothed indicated that the index was increased by about 80 per cent by the full clothing of vest, shirt and coat. It is not conceivable then that differences in the amount of clothing

at different ages would produce changes as great as the metabolism curve necessitates. The reality of these conclusions is substantiated when the trend of metabolism per unit area is examined in other species than the human.

Age curve of metabolism in other species. Brody and his co-workers ('30) have followed the basal and 'resting' metabolism of farm animals from birth to maturity. They found that for dairy and for beef cattle, for pigs (including the results of Deighton), and for chickens the curves are very similar to those for man. The metabolism per unit area, starting at a low level, rapidly rises to a maximum in early life after which it declines slowly to maturity. The maximum occurs at a period which corresponds to the weaning time of the animal (in the chick it is at about 30 to 40 days of age). In the case of the colt, however, there is a continuous decline of the metabolism per unit area from birth. The final decline with increasing size appears to be a general rule, which would be predicted (from the influence of the size factor) if there was physiological and thermal similarity. Table 7 gives the results for different species as far as they are available. The figures must be accepted with a great deal of caution and are cited only to represent the trend of metabolism with growth.

TABLE 7

Metabolism per square meter per day

SPECIES	LEVEL AT BIRTH	MAXIMUM	AGE FOR MAXIMUM, DAYS	FINAL ADULT LEVEL
Cattle	1200	2000	200	1700
Swine	1300	2400	250	1200
Fowl	600	1400	35	920
Horses	2600	No maximum		950
Man	650	1150	1½ years	925

It will be noted that the final decrease to the adult level is considerably less in the case of cattle than in that of swine and of horses. This is what might be expected if this decline is attributable to the increasing amount of insulation, for the high final level of the metabolism per square meter of cattle

indicates that the insulation in their case must be less effective than that of other species. If this is so, increase in the amount of this insulation would not be expected to produce as much change as increase in the amount of more effective insulation such as that of the pig.

Brody ('30) after examination of the metabolism during growth of a number of different species, including men, cattle, sheep, horses, swine, dogs, guinea pigs, rats and some birds, concludes that for all the metabolism per unit weight may be well represented by an equation of the form—

$$Q/m = Ae^{-km} + C$$

where Q is the metabolism per unit time, 'm' is the weight of the animal and A, *K* and C are constants. He also shows from actual measurements of surface area of animals that the surface area follows a law—

$$S = Bm^n$$

where B and n are constants for a particular species, varying of course from one species to another. (Meeh's formula for surface area assumes $n = 2/3$.) Combining the two equations to find the metabolism per unit area instead of per unit weight, we find—

$$H = Q/S = \frac{(Ae^{-km} + C)\, m^{1-n}}{B}$$

Examination of the variation of the quantity H with increasing weight, by the methods of the calculus upon this equation, shows that it will follow just such a curve as has been found to hold for metabolism per unit area, there being an initial rise to a maximum and a subequent more gradual decline. The maximum is predicted by the calculus to occur at a weight given by—

$$m = \frac{1}{k}\left[(1-n) + \frac{C}{A}(1-n)\, e^{1-n}\right]$$

approximately.

It is quite indefensible to use the results of calculations made from empirically fitted mathematical laws unless it is

evident that no extrapolation is involved to regions beyond the experimental range. Thus it cannot be concluded that since the form of the equation for the metabolism per kilogram is a general one for all species, the maximum exists for all species. For instance, for man the constants of the equations given by Brody are $k = 0.035$, $n = 0.7$, $c = 20$, $A = 50$. These give the weight at which the maximum occurs as 13 kg. Actually it is found to be at about 10 kg. Whereas, from the equations given for the horse, $k = 0.008$, $n = 0.63$, $c = 22$, $A = 60$ we find the maximum predicted at a weight of 90 kg., which is far below the range of the experimental values to which the equations are fitted and has therefore no significance. Inasmuch, however, as the form of the equations seems to be generally applicable for the later ages, the eventual decline of metabolism per unit area with increasing size appears to be a general law.

Biological implications; the surface area law. Since Rubner pointed out the constancy among animals of very widely different size and species of the metabolism per unit area, a great deal of discussion and controversy has ensued whether this 'surface area law' has a causal significance, the heat loss determining heat production, or whether it is not to be regarded as a mere coincidence, some other factor as the 'mass of active protoplasmic tissue' of Benedict ('15), which happens to be proportional to the surface area of the animal, is the determining factor. Even the most decided opponents of the causal significance, however, cannot deny that on the average heat loss must be balanced by heat production, if the body temperature is to be maintained.

It may well be argued that the emphasis should be shifted to the consideration of the last phrase; to the fact that of the varying characteristics of growth the body temperature is one that remains at almost unvarying level. The existence of the complicated and very effective mechanism of heat regulation in the homoiotherms for the maintenance of the body temperature within narrow limits suggests that its constancy is something fundamental and essential to the animal.

Van't Hoff's law may apply to isolated chemical processes, but it very evidently does not hold for the complex of linked chemical and nervous processes that forms the whole organism. Metabolism increases with the temperature up to a certain point but beyond a certain limit there is instead a decrease, which, if irreversible, results in death. If metabolism increases in fever, the regulating mechanism eventually increases the heat loss by sweating and vasomotor adjustments so that the temperature is restored to normal. This means that there is a change in the thermal properties of the tissues, of the coefficient of heat transfer from interior to surface, which in the case of fever is temporary, lasting only until the body temperature is restored. If we turn to consider increases in metabolism that result from growth of the animal, we realize that if the accompanying increase in surface area is not adequate, there must be compensating changes, permanent in this case, of the thermal properties of the insulating tissues. The necessity of the elimination of the heat produced in order that the optimum temperature for life may be maintained, links together the thermal properties of the body and its thermal activity, and thus its metabolism. The one is a function, in the mathematical sense, of the other.

Which of these two characteristics, the metabolism or the properties of heat transfer, is the controlling factor and which is the controlled, seems to be a question for philosophy rather than for physiology. The observed facts shown by the 'age curve' of metabolism indicate that at first the metabolism increases very rapidly, even more rapidly than the weight, and that the thermal properties of the body tissues change rapidly to compensate. In later life the specific thermal properties of the tissues remain the same, while the metabolism per unit area decreases in accordance with this fact. Of the three connected variables, the thermal activity of the body, the thermal properties of the body, and its temperature, the last seems from its constancy throughout to be the dominating one. Of the remaining interdependent factors, the second, that of the thermal properties, seems to reach

constancy eventually though not in the early stages of growth. Finally, when growth has ceased at maturity, all three factors remain relatively constant.

SUMMARY

The fundamental law of heat flow demands that there be an internal 'physiological' gradient of temperature from the interior of the body to an area of surface as well as an external physical gradient from that surface to the surroundings. The laws governing flow of heat down these gradients are considered. Newton's law applies to the loss of heat from the skin by convection, radiation and conduction, the loss by evaporation being considered separately. The application of the laws of conduction to the transport of heat through the underlying tissues to the skin is shown and the conclusion is reached that the distribution of temperature with depth will be in general, as it has been found, parabolic. A 'thermal circulation index' is defined by which from measurements of skin temperature, changes in the properties of heat transfer of the tissues may be studied. Examples of such changes are given. The dependence of the skin temperature upon internal temperature, upon environmental temperature, and upon the circulation is deduced.

On the assumption that there is thermal 'similarity,' i.e., that the factor of absolute size is the only variable in growth, a relation is deduced for the variation of metabolism per unit area with increasing weight. Comparison with the 'age curves' of metabolism in humans shows that the eventual decrease from an early maximum to the adult level is consistent with an increase in absolute amount of the thermal insulation, its relative amount remaining constant. From birth to the maximum at $1\frac{1}{2}$ years, however, there must be a progressive decrease in the relative amount or in the effectiveness of the insulation.

The trends of metabolism per unit area in other species are discussed and conclusions are drawn as to the interpretation of the surface area law in view of the fact that the body

temperature, the thermal properties of the tissues, and the thermal activity of the body are interdependent.

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POSSIBLE SOURCES OF CALCIUM AND PHOSPHORUS IN THE CHINESE DIET

I. THE DETERMINATION OF CALCIUM AND PHOSPHORUS IN A TYPICAL CHINESE DISH CONTAINING MEAT AND BONE

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That milk is used only for infants and invalids is not merely a prejudice but a dietary practice quite universal among the Chinese. The absence of milk and dairy products in the native Chinese diet has drawn continuous interest because of the sharp contrast in the diet of the occidental world where milk is used freely. This fact has led to an inquiry regarding the source of calcium particularly in the Chinese diet. Soy bean and some of its products have been found to be high in calcium and phosphorus. Definite information is lacking as regards the physiological utilization of soy bean as the main source of supply of these elements. Pittman ('32) has found that even an intake of 4 ounces, dry weight, of navy beans daily was unable to maintain a calcium balance. However, Adolph and Chen ('32) have reported that an equal calcium intake supplied by cows' milk or soy bean curd produced the same effect on calcium equilibrium for the Chinese adult. In addition to the liberal supply of vegetables usually provided the Chinese use fowl, pork and fish as commonly in the diet as Americans use milk and cheese. Recognizing the low content of calcium in meat in contrast to milk, the question arises as to the availability of calcium from the bone in such meat dishes as effected by the method of preparation and way of eating.

A commonly used, cheap dish, 'sweet-sour pork spareribs,' may serve as an illustration. Spareribs are cut up in 1½-inch pieces, slightly browned and cooked slowly until very tender with a large amount of diluted vinegar, soy bean sauce, and sugar to taste. The excess liquid is thickened with starch before serving. Due to the long period of cooking, the solution penetrates the soft bone marrow. It is a satisfaction for those who dine with the family to suck and chew the small pieces of bone while eating. A similar dish but with a large proportion of bones, usually pig's feet, ginger and a high concentration of vinegar is prepared for the lactating mother as a main dish in three or four meals a day, together with eggs, a few vegetables, and occasionally other meats as supplements. This diet is started right after childbirth and lasts 30 to 40 days. It is generally believed that this is good for milk production, blood regeneration and general vitality. Such a time-honored custom, while it appears to lack any scientific support, seems worth investigation to determine the actual amounts of calcium and phosphorus obtained from the consumption of such a combination of bone, meat and sauce.

This study is devoted only to the quantitative measure of the possible amounts of calcium and phosphorus obtained from this particular preparation of meat and bone. The forms in which these elements might be found were not determined nor were biological metabolic tests made to determine the absorption and utilization of such elements in the human or animal body.

EXPERIMENTAL

Standardization of the preparation of 'sweet-sour spareribs.' To provide a typical Chinese meat-bone dish for analysis, the recipe and method of cooking were tested until the finished product resembled the native home-made dish. The standardized formula for an individual serving is as follows:

Pork spareribs	150 to 180 gm.
Cooking solution (total)	250 cc.
Rice-vinegar	75 cc.
Soy bean sauce	10 cc.
Distilled water	150 cc.
Sugar	10 gm.
Salt	1 gm.
Thickening	
Cornstarch	5 gm.
Distilled water	15 cc.

Pork is the only kind of meat used in this dish. To make it more typical, imported Chinese rice-vinegar and soy bean sauce were used. This vinegar is an acid product resulting from the fermentation of rice. Soy bean sauce is well known as the chief seasoning in Chinese cookery. Since it is made from fermented soy beans the question has often been raised whether or not it furnishes additional calcium and phosphorus in the Chinese diet.

Sampling. In preparing samples for this experiment, the spareribs of one side of the animal were purchased from the market. The middle six ribs were chosen and each cut into six 1½-inch pieces. Alternate pieces of each rib were taken to make two rather uniform samples. One of these samples was cooked in a small enameled kettle, over an electric plate, starting with a medium temperature. The meat was browned slightly and then the cooking solution was added, covered and cooked at low temperature with little stirring until the meat was tender and came readily off the bone. This took from 50 to 60 minutes. The remaining liquid was thickened with the cornstarch paste. After it boiled again the heat was turned off and the contents allowed to stand for 15 minutes, the time considered used in waiting to be served. The total time for preparation was 80 minutes, 75 of which the meat and bone were in contact with the solution. The meat was separated from the bone with a knife and fork, then washed with hot distilled water and drained. All visible pieces of bone were collected and treated likewise. The solution together with the washings of the meat, bone and untensils was collected. For the raw sample, the bone was separated as

completely as possible from all the meat fibers. Below is a list of samples taken for analysis in the preparation of the prepared meat-bone dish.

Bone series	*Meat series*	*Solution series*
Raw bone	Raw meat	Soy bean sauce
		Rice-vinegar
Cooked bone	Cooked meat	Cooking solution
		Cooking solution (cooked)

Methods of calcium and phosphorus determinations. The calcium was determined by precipitation as calcium oxalate and titration with standard $KMnO_4$ solution. The precipitation was made in the presence of ammonium chloride, in a hot solution which had been made pink to methyl red by the addition of acetic acid. The calcium oxalate was titrated under conditions suggested by the U. S. Bureau of Standards ('20 and '12) in which the standardization of $KMnO_4$ solution was made with sodium oxalate.

Phosphorus was determined by Pemberton's alkalimetric method (1895). The phosphorus was precipitated as ammonium phosphomolybdate. The yellow precipitate was dissolved in an excess of standard NaOH solution. The excess NaOH was back titrated with standard HCl. This is similar to a method given by the Association of Official Agricultural Chemists ('30).

RESULTS

Calcium and phosphorus in the cooking solution. To check the possible sources of calcium and phosphorus the rice-vinegar, soy bean sauce and the combination of all the ingredients in the cooking solution used in the recipe were analyzed for these elements. The results are recorded in table 1. The total calcium of the cooking solution was only 2 mg. more than the sum of the calcium from vinegar and soy bean sauce. The total phosphorus was somewhat higher than the sum of the quantities in the two ingredients. Regardless of this fact, the actual amounts of calcium and phosphorus in the cooking solution were taken into consideration in the final results.

The titratable acidity of the cooking solution was determined, giving a normality range of 0.150 to 0.152. The pH value as measured by means of a hydrogen electrode was 3.20 to 3.24.

Solubility of tricalcium phosphate in water and cooking solution. Four samples of tricalcium phosphate were weighed out. Two of these samples were cooked in 167 cc. of the cooking solution, as described in the recipe, for 1 hour. The others were kept in the same amount of water as the cooking solution for the same length of time. A clear solution for the determination of solubility was obtained by centrifuging. The results are shown in table 2. The percentages of solubility

TABLE 1

Calcium and phosphorus in the cooking solution

INGREDIENTS	SAMPLE	CALCIUM	AVERAGE CALCIUM	PHOSPHORUS	AVERAGE PHOSPHORUS
	cc.	gm.	gm.	gm.	gm.
Rice-vinegar 1	75	0.003		0.007	
Rice-vinegar 2	75	0.003	0.003	0.006	0.006
Soy bean sauce 1	10	0.022		0.008	
Soy bean sauce 2	10	0.023	0.023	0.008	0.008
Cooking solution 1	25	0.003		0.005	
Cooking solution 2	25	0.003	0.003	0.005	0.005
Cooking solution in recipe	250		0.028		0.048

were estimated on the basis of the calculated weights of calcium and phosphorus in the tricalcium phosphate samples. The percentage of solubility of each element in the cooking solution was decidedly higher than in the water. Taking the highest percentages in all cases, calcium gave 8.09 per cent solubility in water and 30.60 per cent in the cooking solution, whereas the phosphorus showed 6.43 per cent solubility in water and 14.47 per cent in the cooking solution. The results agree with the statement of Cameron and Seidell ('04), that tricalcium phosphate is only slightly soluble in water but more soluble in a dilute acid medium. Comey ('28) found that very small quantities of salts of alkali metals increase the solubility of tricalcium phosphate in water, and that it

is also more soluble when the water contains starch, glue or other animal substances. According to such statements, this cooking solution provides conditions favoring the solubility of tricalcium phosphate. While there was considerable difference in solubility of calcium and phosphorus in samples I and II, however, the cooking in the vinegar-soy bean sauce solution made soluble larger quantities of these elements.

Loss of calcium and phosphorus from bone after cooking in vinegar-soy bean sauce solution. Since it has been shown that the solubility of tricalcium phosphate is greater in acid

TABLE 2

The solubility of $Ca_3(PO_4)_2$ in water and cooking solution[1]

SOLUTIONS	$Ca_3(PO_4)_2$ IN SAMPLE	CALCULATED AMOUNT OF CA IN SAMPLE	CALCULATED AMOUNT OF P IN SAMPLE	AMOUNT OF CA OBTAINED FROM SAMPLE	AMOUNT OF P OBTAINED FROM SAMPLE	PER CENT SOLUBILITY OF CA	PER CENT SOLUBILITY OF P
	gm.	*gm.*	*gm.*	*gm.*	*gm.*		
Water 1	0.842	0.326	0.516	0.026	0.033	8.09	6.43
Water 2	1.603	0.620	0.983	0.027	0.063	4.38	6.39
Cooking solution 1	1.297	0.502	0.795	0.154	0.105	30.60	13.19
Cooking solution 2	1.633	0.632	1.001	0.190	0.145	30.12	14.47

[1] The amounts of Ca and P in the cooking solution were subtracted from the total amounts of these elements obtained from the $Ca_3(PO_4)_2$ after it was cooked in the solution.

solution than in water, it may be assumed that there would be larger amounts of calcium and phosphorus dissolved from bone cooked in acid medium than in water. Bone samples were prepared as described. In this particular determination the meat was removed from the bone and weighed before cooking. After the cooking process, all visible pieces of bone were collected for analysis. The results are shown in table 3. The difference in the percentage of calcium and phosphorus in the raw and cooked bone samples might appear to be insignificant but it is constant and a certain loss resulting from cooking may be assumed. From the mathematical calculation, based on the percentages of the calcium and phosphorus in

the raw bone (table 3), a sample of 45.40 gm. of bone should yield 4.354 gm. of calcium and 2.202 gm. of phosphorus. Comparing this theoretical yield with the actual amounts of 3.988 gm. of calcium and 2.058 gm. of phosphorus obtained from the cooked sample, a loss of 0.366 gm. calcium and 0.144 gm. phosphorus is evident.

Calcium and phosphorus in the meat-bone dish. From the above, it seemed possible to interpret the results obtained from the prepared meat-bone dish. Analytical data for the meat and the solution after cooking are shown in tables 4

TABLE 3

Loss of calcium and phosphorus in bone after cooking

BONE SAMPLE	TOTAL MATERIAL	RAW BONE	ASH	CALCIUM	PHOSPHORUS	PER CENT IN RAW BONE	
						Calcium	Phosphorus
			gm.	*gm.*	*gm.*		
Raw 1			5.339	2.043	1.025		
Raw 2			5.072	1.906	0.964		
Total	151.50	41.20	10.411	3.949	1.989	9.59	4.85
Cooked 1			5.271	1.965	1.009		
Cooked 2			5.514	2.023	1.049		
Total	165.30	45.40	10.785	3.988	2.058	8.78	4.53
Theoretical yield from 45.4 gm. bone				4.354	2.202		
Loss in 45.4 gm. of bone after cooking				0.366	0.144	— 0.81	— 0.32
Loss in 100 gm. of bone after cooking				0.806	0.318		

and 5. The bone samples were also analyzed. Since there was no way to get the separate weight of the raw bone and meat, which were supposed to be cooked together according to the Chinese method, no comparison of the percentage of calcium and phosphorus in these bone samples could be made. During cooking, considerable amounts of the organic materials of the bone dissolved, lessening the net weight of the bone. As a result, a higher percentage of ash in the total weight of the bone is found in the cooked sample than in the raw bone. Therefore, the data of the bone analyses were not included in the interpretation of the yields of calcium and phosphorus from this meat-bone dish. The determinations of

calcium and phosphorus in both raw and cooked meat are found in table 4. From nearly equal amounts of raw material, the meat after it was cooked had an extracted dry weight about two times greater than that of the raw. Consequently, the ash of the cooked meat was about four times greater than the raw, in spite of the fact that considerable amount of the tissue fibers had gone into the solution, as a result of long cooking. The comparison of the amounts of calcium and phosphorus are equally striking. In the cooked meat sample, there were 0.451 gm. of calcium and 0.270 gm. of phosphorus as compared to 0.028 gm. of calcium and 0.098 gm. phosphorus in the raw meat sample. Assuming that the meat from nearly

TABLE 4

Comparison of calcium and phosphorus in meat (raw and after cooked in solution)

SAMPLES	MEAT AND BONE	EXTRACTED MEAT	TOTAL ASH	CALCIUM IN ASH	PHOSPHORUS IN ASH	PER CENT IN ASH	
						Calcium	Phosphorus
	gm.	*gm.*	*gm.*	*gm.*	*gm.*		
Raw meat 1		6.585	0.253	0.016	0.056	6.44	22.28
Raw meat 2		6.159	0.162	0.012	0.042	7.09	25.74
Total	172.9	12.744	0.415	0.028	0.098	6.69	23.63
Cooked meat 1		13.161	1.131	0.299	0.176	26.43	15.54
Cooked meat 2		9.060	0.556	0.152	0.094	27.30	16.91
Total	173.8	22.221	1.687	0.451	0.270	26.71	13.99

equal weights of the corresponding parts of the same piece of spareribs was equal, a considerable gain of these minerals was obtained as a result of cooking. Since no reported studies could be found regarding the possibility of absorption of calcium and phosphorus by meat during the cooking process, it is assumed that these elements, after being dissolved from the bone must be accounted for in the meat and cooking solution. Another possible assumption could also be made. Due to the salt-forming property of the proteins, calcium may be taken up to form calcium proteinate. The amounts of calcium and phosphorus obtained from the solution after the meat and bone are cooked in it are given in table 5. The calcium in the cooked solution was 0.100 gm. and the phosphorus 0.114

gm., as against 0.028 gm. of calcium and 0.048 gm. of phosphorus in the solution before cooking, thus indicating a gain of 0.072 gm. calcium and 0.066 gm. phosphorus. The reason why the amounts of calcium and phosphorus are not higher is well explained by the previous assumptions that considerable quantities may adhere to the meat or form calcium proteinate.

TABLE 5

Comparison of calcium and phosphorus in solution before and after cooking

SOLUTION	AMOUNT USED	ASH	CALCIUM IN ASH	PHOSPHORUS IN ASH
	cc.	*gm.*	*gm.*	*gm.*
First portion after cooking		1.937	0.047	0.050
Second portion		2.334	0.053	0.064
Total	250	4.271	0.100	0.114
Solution before cooking	250		0.028	0.048
Gain after cooking			0.072	0.066

TABLE 6

Distribution of the measurable amounts of calcium and phosphorus in an individual serving of 'sweet-sour-spareribs'

SOURCE	RAW MATERIAL	CALCIUM	PHOSPHORUS	CA : P
		gm.	*gm.*	
Meat and bone	173.8 gm.	0.451	0.270	
Cooked solution	250 cc.	0.100	0.114	
Total		0.551	0.384	1.43

There appears to be sufficient evidence to conclude that in the process of cooking there is a definite loss of calcium and phosphorus from the bone with a relative increase in these minerals in the meat and the cooking solution. The distribution of the measurable amounts of calcium and phosphorus actually obtained from an individual serving of 'sweet-sour-spareribs' is given in table 6. In a 173.8 gm. (about 6.5 ounces) sample of meat and bone, cooked with the diluted vinegar and soy bean sauce solution, according to the method described, 0.551 gm. of calcium and 0.384 gm. of phosphorus are obtained.

DISCUSSION

The increased amounts of calcium and phosphorus in the meat and solution after cooking furnish proof that the increase comes from the bone. Also, the comparative amounts of calcium and phosphorus of the crude bone, raw, and after cooking, give further proof of the loss of these elements from the bone. After cooking, a 45.4-gm. sample of raw bone from 165.3 gm. of spareribs showed a loss of 0.366 gm. calcium and 0.144 gm. phosphorus. From a slightly larger amount of spareribs, namely 173.8 gm., it may be assumed that the weight of the raw bone would be somewhat higher and, therefore, the total loss of these elements would be greater. The loss can be accounted for when we compare the gain of calcium and phosphorus in the meat and solution after cooking (tables 4 and 5).

It thus appears from the data on the meat and solution after cooking that this one individual serving of 'sweet-sour-spareribs' composed of 173.8 gm. of bone and meat with 250 cc. of the vinegar and soy bean sauce solution, cooked at low temperature for 1 hour, furnished 0.551 gm. of calcium and 0.384 gm. of phosphorus. These amounts gave a Ca : P ratio of 1.43. Although the amounts of calcium and phosphorus obtained give a ratio believed to be favorable for assimilation (Daniels and Hutton, '28), the forms in which those elements exist and the physiological availability for human utilization require further experimental study. According to Sherman ('32) the daily allowance for the average man is 0.68 gm. of calcium and 1.32 gm. of phosphorus. When these elements are adequately furnished, the forms and ratio in which they are supplied are of less importance.

If these amounts of 0.551 gm. of calcium and 0.384 gm. phosphorus obtained from this 'sweet-sour-spareribs' dish were assimilated as satisfactorily as the well-recognized sources, such as milk and cheese, this special way of cooking meat and bone is one which should be favored and used plentifully in the diet, especially when milk is not provided in adequate amounts.

SUMMARY AND CONCLUSION

1. In the Chinese diet, milk and cheese, the well-recognized sources of calcium and phosphorus, are not used. A typical Chinese dish, which is called 'sweet-sour-spareribs,' was analyzed as a possible source of these elements. Pork spareribs were cut into 1½-inch pieces and cooked in a rice-vinegar, soy bean sauce, salt and sugar solution for 1 hour at a low temperature. Samples of bone, meat and cooking solution were analyzed for calcium and phosphorus before and after cooking.

2. Tricalcium phosphate is found to be more soluble in the cooking solution with a pH of 3.2 containing salt and sugar, than in water. Providing the calcium and phosphorus of bone exist in large proportion as tricalcium phosphate, this cooking solution would favor its solubility.

3. From the analysis of a sample of 173.8 gm. of meat and bone, total amounts of 0.551 gm. of calcium and 0.384 gm. of phosphorus were obtained. Of these amounts, 0.451 gm. calcium and 0.270 gm. phosphorus were obtained from the meat, and 0.100 gm. calcium and 0.114 gm. phosphorus from the solution, after cooking. In this particular brand of soy bean sauce, only traces of calcium and phosphorus were found, while those of the rice-vinegar were negligible.

4. Quantitatively, the amount of calcium obtained in this dish exceeds the minimum requirement of 0.45 gm., and approaches the allowance of 0.68 gm. per man per day, while the amount of phosphorus obtained hardly reaches half the minimum requirement. The latter, however, is more generously distributed in foods and it is easier, therefore, to fulfill the daily requirement. Biological experiments should be carried on in animals and humans to find out how these elements from such a source are metabolized. If they are absorbed and utilized satisfactorily, another significant source of calcium and phosphorus has been demonstrated.

5. It is possible that this peculiar method of cookery used extensively by the Chinese may be of particular value in providing adequate amounts of calcium and phosphorus in the Chinese diet. Also the Chinese method of chewing and sucking small pieces of bones while eating may increase the total amounts of these elements.

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HYPERTHYROIDISM AND NUTRITION

I. VITAMIN B AND THYROXIN[1]

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EIGHT FIGURES

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The protective influence of various foodstuffs in experimental hyperthyroidism (rat) has been investigated during the last 10 years by Abelin and associates ('30) who found that the basic metabolic rate produced by excessive amounts of desiccated thyroid or thyroxin can be materially reduced and liver injury prevented to a great extent by feeding large amounts of pure casein, yeast and egg yolk, and by certain glandular organs, such as kidney.

That the vitamin B requirement of the animal organism is determined chiefly by its calorific requirement has been pointed out in 1927 by Plimmer, Rosedale and Raymond ('27) and by Cowgill and Klotz ('27).

Himwich, Goldfarb and Cowgill ('31) reported an increase in the requirement of undifferentiated vitamin B of the dog during intervals of feeding desiccated thyroid, as determined by the period of onset of anorexia and loss of body weight. Recently Cowgill and Palmieri ('33), using desiccated thyroid and Harris yeast as a source of vitamin B, found similar results with pigeons, that is, the vitamin B requirement proved to be greater in hyperthyroidism than under 'normal' conditions.

[1] Research paper no. 302, Journal series, University of Arkansas.

Recently von Euler and Klussmann ('32) observed that rats on a diet deficient in vitamin A and injected with thyroxin suffered loss of weight less rapidly when also receiving a daily dose of carotin (or carotene).

EXPERIMENTAL

In this investigation a study was made of the toxicity of thyroxin in the albino rat and of the protective influence of the vitamin B complex and vitamin B against the injury produced by thyroxin. A highly concentrated vitamin B extract was used, prepared by one of us (B. S.), which is 20,000 times as potent in vitmin B as cow's milk (Sure,'32). Three to 5 mg. of the extract, when given orally daily, resulted in a growth of 12 to 15 gm. weekly per animal. The thyroxin employed was a pure crystalline product secured from E. R. Squibb & Sons.

The results of our findings are summarized in charts 1 to 8 inclusive. In all this work litter mates of the same sex were employed.

Toxicity of thyroxin on diets abundant and deficient in the the vitamin B complex

It is apparent from charts 1 and 2 that the albino rat can tolerate large amounts of thyroxin when taken in by mouth, ♂ 9638 having survived 19 days and ♂ 9635 a period of 36 days on as much as 1 mg. daily. Male no. 9637, however, survived only 5 days on the same daily dosage, but this animal was probably already injured by cumulative effects of the smaller dosages which it received during the previous 3 weeks.

From charts 3 and 4, it will be noted that 0.01 to 0.02 mg. thyroxin given daily by mouth produced no deleterious effect on the rat as judged by changes in body weight, on a ration (no. 1845) abundant in the vitamin B complex, of the following composition: casein (purified), 20; salts no. 185, 4 (McCollum and Simmonds, '18); Northwestern yeast (baker's, dehydrated), 10; dextrin, 56.

It is evident from chart 3 that on ration 1845 no decline in weight has occurred following subcutaneous injections of 0.25 mg. thyroxin daily for a period of 12 days. When, however,

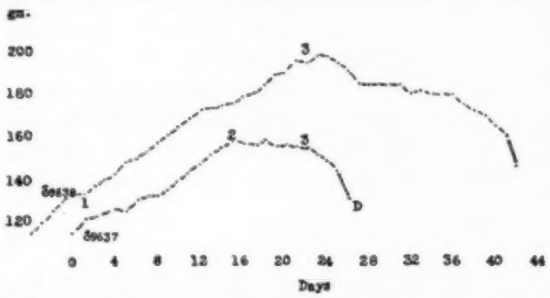

Chart 1, showing toxicity of thyroxin administered orally, supplementing a ration abundant in the vitamin B complex.

1. 0.1 mg. thyroxin daily.
2. 0.2 mg. thyroxin daily.
3. 1.0 mg. thyroxin daily.

D=Died

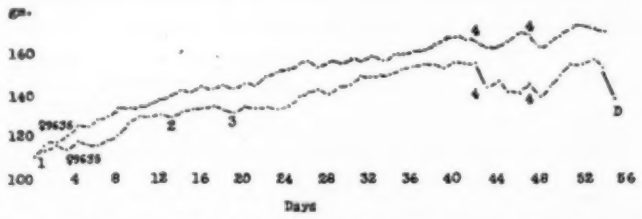

Chart 2, showing toxicity of thyroxin administered orally, supplementing a ration abundant in the vitamin B complex.

1. 0.1 mg. thyroxin daily.
2. 0.2 mg. thyroxin daily.
3. 1.0 mg. thyroxin daily.
4. Fasted 18 hours previous to sampling for blood cholesterol.

D=Died

the ration was changed to one deficient in the vitamin B complex (no. 1751), ♀ 9614 that received no thyroxin survived with a loss of 30 gm., while its litter mate died on the thirteenth day of the vitamin depletion period, with a loss of 50 gm. of body weight.

Chart 4 shows the toxic effect of 0.25 mg. thyroxin administered subcutaneously six times a week. Male no. 9615 stopped growing on ration 1865 as soon as the parenteral injections of thyroxin were begun and survived 23 days. Its

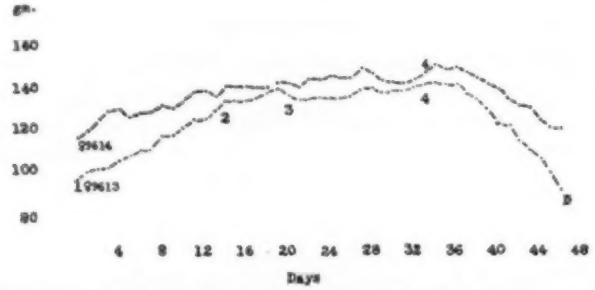

Chart 3, showing toxicity of thyroxin administered subcutaneously, on a ration abundant, which was later changed to one deficient in the vitamin B complex.

1. 0.01 mg. thyroxin daily, orally.
2. 0.02 mg. thyroxin daily, orally.
3. 0.25 mg. thyroxin subcutaneously daily, with the exception of Sundays.
4. Ration changed so that it was deficient in the vitamin B complex.

D=Died

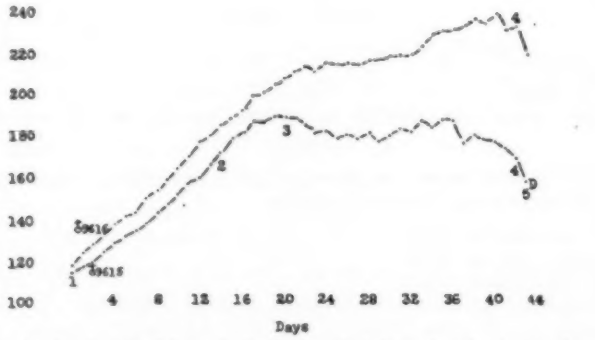

Chart 4, showing toxicity of thyroxin administered subcutaneously, supplementing a ration abundant in the vitamin B complex.

1. 0.01 mg. thyroxin daily, orally.
2. 0.02 mg. thyroxin daily, orally.
3. 0.25 mg. thyroxin subcutaneously daily, with the exception of Sundays.
4. Fasted 18 hours previous to sampling for blood cholesterol.
5. Died while obtaining blood sample peripherally..

litter mate, ♂ 9616 served as a control, which made continuous growth on the same ration, without thyroxin administration.

From chart 5 it is apparent that as much as 0.25 mg. thyroxin administered subcutaneously for 10 days had no noteworthy catabolic effect on ♀ 9617, since there were no losses in body weight. When, however, the diet of this animal as well as that of its litter mate was depleted of the vitamin B complex, striking differences in results were obtained. Female no. 9617, that received the thyroxin, died in 11 days, accompanied by a loss of 47 gm. in body weight, while its litter

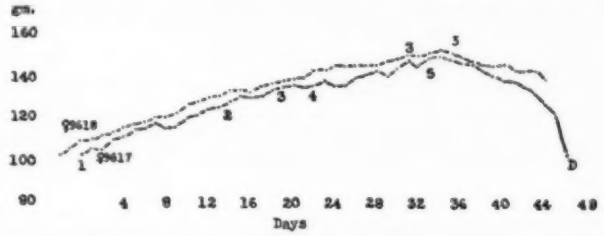

Chart 5, showing toxicity of thyroxin administered subcutaneously, on a ration abundant, which was later changed to one deficient in the vitamin B complex.

1. 0.02 mg. thyroxin daily, orally.
2. 0.04 mg. thyroxin dailly, orally.
3. Thyroxin removed.
4. 0.5 mg. thyroxin subcutaneously daily, with the exception of Sundays.
5. Ration changed so that it was deficient in the vitamin B complex.

D=Died

mate, ♀ 9618, that received no thyroxin, survived, with a loss of only 11 gm.

Male no. 9619 (chart 6) survived for 23 days, following injection of as high as 0.5 mg. thyroxin daily, six times a week, on our ration 1865, containing an abundance of vitamins B and G in the form of dried baker's yeast.

Protective influence of the vitamin B complex against toxicity of thyroxin

It is evident from chart 7 that a daily oral administration of 0.08 to 0.16 mg. thyroxin for a period of 19 days apparently produced a depressing effect on the growth of ♂ 9634, since

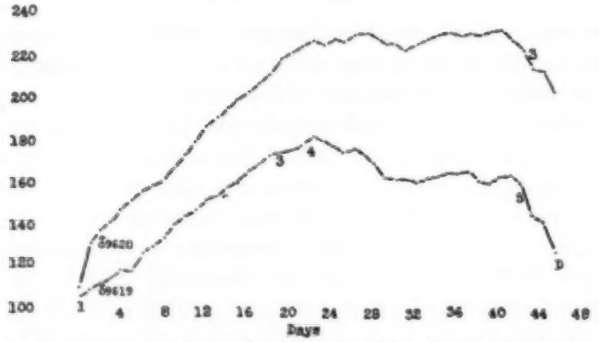

Chart 6, showing toxicity of thyroxin administered subcutaneously, supplementing a ration abundant in the vitamin B complex.

1. 0.02 mg. thyroxin daily, orally.
2. 0.04 mg. thyroxin daily, orally.
3. Thyroxin removed.
4. 0.5 mg. thyroxin subcutaneously daily, with the exception of Sundays.
5. Fasted for 18 hours previous to sampling blood for cholesterol.

D=Died

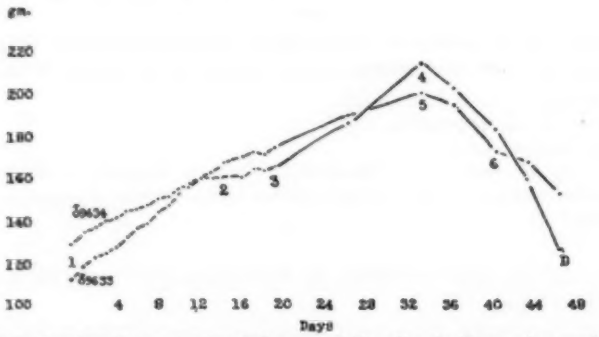

Chart 7, showing the protective influence of the vitamin B complex against the toxicity of thyroxin.

1. 0.08 mg. thyroxin daily, orally.
2. 0.16 mg. thyroxin daily, orally.
3. Thyroxin removed.
4. 1.0 mg. thyroxin daily, orally, and ration changed so that it was deficient in the vitamin B complex.
5. 1.0 mg. thyroxin daily, orally, ration depleted of the vitamin B complex, and 12 mg. of vitamin B concentrate administered daily separately from the ration.
6. Vitamin B concentrate increased to 24 mg. daily.

D=Died

the removal of thyroxin during the subsequent 14 days was followed by a much more marked rate of growth. The noteworthy fact, however, in this experiment became apparent when a daily oral dose of 1 mg. was begun on the thirty-third day of the experiment. This daily dose was administered to both animals 9633 and 9634. At the same time the ration was depleted of the vitamin B complex. Male no. 9634, however, was given separately from the ration 12 mg. of a highly concentrated vitamin B preparation, which was increased 7 days later to 24 mg. Male no. 9633 died on the thirteenth day of the vitamin depletion period with a total loss of 110 gm., or 52 per cent of its body weight, while ♂ 9634, which was placed on the same ration depleted of the vitamin B complex, and which received the same daily dose of thyroxin, but because it received a vitamin B concentrate during this experimental period, survived with a loss of only 47 gm., or 23.5 per cent of its body weight.

Protective influence of vitamin B against toxicity of thyroxin

Chart 8 indicates that a daily oral dose of 0.03 to 0.06 mg. thyroxin for 21 days produced no noteworthy depressing effect on the growth of ♀ 9623. A daily subcutaneous dose of 0.25 mg.,[2] however, on a ration (no. 2345) deficient only in vitamin B[3] (Sure, '33) was followed by a total collapse of the animal in 30 days, accompanied by a loss of 98 gm., or 54 per cent loss of body weight; while litter mate, ♀ 9624 that was subjected to the same treatment, but because it received during the experimental period 6 to 18 mg. daily of a highly potent vitamin B concentrate, survived with a loss of only 26 gm., or 16 per cent loss of body weight.

The several blood cholesterol determinations made on pathological and control animals indicate a hypocholesteremia during periods of hyperthyroidism. This point we do not consider, however, as yet established until we have corroborated such findings on a large number of animals.

[2] Daily with the exception of Sundays.

[3] Composition of ration 2345 is as follows: Casein (purified), 10; autoclaved round beef steak, 15; salts no. 185, 4; butter fat, 10; dextrin, 61.

Necropsy findings of animals that succumbed from hyperthyroidism showed slight thoracic hemorrhages, peripheral and venous constriction and arterial dilatation. The heart and blood vessels, particularly veins, were filled with blood. The

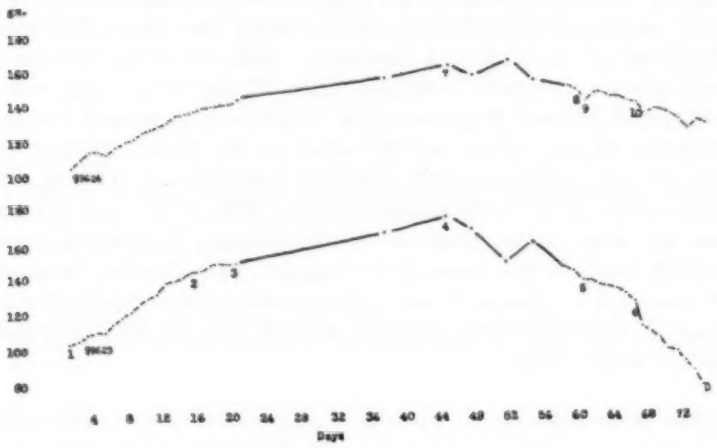

Chart 8, showing the protective influence of vitamin B against the toxicity of thyroxin.

1. 0.03 mg. thyroxin daily, orally.
2. 0.06 mg. thyroxin daily, orally.
3. Thyroxin removed.
4. 0.5 mg. thyroxin subcutaneously daily, with the exception of Sundays, and ration depleted of vitamin B.
5. Bled peripherally for blood cholesterol.
6. Fasted for 18 hours previous to sampling for blood cholesterol.

D = died.

7. 0.5 mg. thyroxin subcutaneously daily, with the exception of Sundays, ration depleted of vitamin B, and 6 mg. of vitamin B concentrate daily administered separately from the ration.
8. Increased vitamin B concentrate to 18 mg. daily.
9. Bled peripherally for blood cholesterol.
10. Fasted for 18 hours previous to sampling for blood cholesterol.

heart appeared hypertrophied and fibrous on macroscopic examination. In one case the thyroid was enlarged.

Since excessive amounts of thyroxin stimulate catabolism of body tissues and particularly injury of the liver (Abelin,

'30, Abelin, Knochel and Spichtin, '30) which is the storage depot of vitamin B, it is of course to be expected that during the process of tissue oxidation the vitamin B will be at the same time destroyed; hence, the greater requirement of this vitamin the more thyroxin is made available in circulation.

A study of a quantitative balance between amount of thyroxin and vitamin B concentrate is in progress, the results of which will be reported later.

The fact that in experimental hyperthyroidism protection can be afforded by a highly concentrated vitamin B preparation, would suggest that oral or preferably parenteral administrations of potent standardized vitamin B concentrate may be indicated in toxic goiter, particularly in non-operative cases. This is, however, a problem for the clinician to solve.

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THE STORAGE OF VITAMIN A IN THE LIVER OF THE RAT [1]

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ONE FIGURE

(Received for publication July 25, 1933)

INTRODUCTION

In the course of a prolonged study of the effects of isolated radiations of equal energy in the visible and infra-red regions of the spectrum upon the growth and development of normal rats, the following results, though in the nature of a side issue to the main problem, seem to merit publication.

In such a study, our method has been to raise rats on a standard normal diet, and to vary nothing in the experiment, except the isolated band of radiation to which the rat has been exposed. The diet chosen for this purpose was the well-known Sherman B diet (Sherman and Campbell, '24), which consists of dried whole milk one-third, ground whole wheat two-thirds, NaCl 2 per cent of the wheat. In the course of our experiments we observed at autopsy the occurrence of spontaneous middle ear infections occurring in a small percentage of our older rats. This infection did not appear to interfere with the general health of the animals, but we had evidence that it produced a slight limitation of growth as compared with the growth of non-infected animals. In view of the many claims for the anti-infective nature of vitamin A we con-

[1] Aided by a grant from the Committee on the Effects of Radiation upon Living Organisms, Divisions of Biology and Agriculture, National Research Council.

sidered the adequacy of the vitamin A content of the diet and decided to study it by chemical methods. In this diet the supply of vitamin A comes mainly from dried whole milk, whole wheat being a poor source of this factor. Milk is known to be a variable source of vitamin A, its potency in this factor depending upon the diet of the cow, and being subject to seasonal variations. In addition to this, the possibility of the destruction of vitamin A during the process of evaporation would tend to decrease its value.

We decided to make chemical determination of the vitamin A content of the blood, livers, and body fat of a series of our animals, with and without the addition of vitamin A to the diet.

Rosenheim and Webster ('27) observed that halibut liver oil was a much more potent source of vitamin A than cod liver oil. This observation has since been abundantly confirmed. Halibut liver oil has the additional advantage of containing very little vitamin D, so for these reasons we used it as a source of additional vitamin A in our experiments. The rats used in these experiments were of The Wistar Institute Experimental Colony strain. The mothers were shipped to us during the latter part of their pregnancy. The young were reared after weaning on the Sherman B diet.

Technic employed for the collection of samples

Blood. The rat was anaesthetized with illuminating gas. It was then bled rapidly from the heart, and the blood collected into a small test tube of 10 ml. capacity. When clotting had occurred the serum was analyzed for vitamin A by the method described below.

Liver. The abdomen was opened and the entire liver carefully removed. After this organ had been freed from adherent tissue, and any excess of moisture or blood removed with clean filter paper, it was weighed accurately to 0.01 gm. It was then analyzed for vitamin A by the method described below.

Body fat. Both perinephric and mesenteric fat were taken from the rat, and a mixed sample of these used in making the tests.

METHOD

Reagents

With the exception of the alcohol, which was the pure undenatured 95 per cent alcohol obtained from the American Commercial Alcohol Company, all the chemicals described below were Baker's C.P. analyzed quality.

Chloroform. It is essential that the chloroform used should be as nearly anhydrous as possible and free from alcohol. To insure this Baker's C.P. chloroform was thoroughly washed with a large excess of distilled water, dried with anhydrous calcium chloride, and distilled, by means of a Clarke's fractionating column (Clarke and Rahrs, '23) over anhydrous calcium chloride.

Antimony trichloride reagent. To approximately 300 cc. of the anhydrous chloroform, 100 gm. of antimony trichloride were added. After the chloroform was well saturated the excess of the salt was filtered off, and the clear solution stored in a tightly stoppered brown glass bottle.

Copper sulfate standard solution. Ten grams of copper sulfate ($CuSO_4.5H_2O$) were dissolved in 90 cc. of distilled water.

Fifty per cent ethyl alcohol. To 500 cc. of the 95 per cent ethyl alcohol 500 cc. of distilled water were added.

Determination of vitamin A

Blood. The tube containing the clotted blood was centrifuged. Two cubic centimeters of clear serum were placed in a special tube with a narrow neck of 10 ml. capacity, 2 cc. of 95 per cent ethyl alcohol added, and the contents well mixed; finally 2 cc. of petroleum ether were introduced and the tube was shaken at intervals for 10 minutes. It was then centrifuged and 1 cc. of the clear layer of petroleum ether transferred to a 10 ml. test tube. This tube was placed in a beaker

containing water at 40 to 45°C., the petroleum ether was rapidly evaporated by the introduction of a gentle current of dry air into the tube. The residue was dissolved in 0.1 cc. of the anhydrous chloroform, 1 cc. of the antimony trichloride reagent was added, and the intensity of the resulting blue color determined 30 seconds after mixing in a colorimeter, using the copper sulfate solution as a standard. By definition, 1 arbitrary unit of vitamin A is said to be present when standard and unknown match exactly. If, therefore, the standard and unknown match in the case of serum or plasma, the vitamin A units of the sample will be 100 units per 100 cc. This unit is approximately 0.01 Sherman unit.

Liver. One gram of liver (weighed to 0.01 gm.) was transferred to a small mortar, fine sharp sand was added and 5 cc. of the 50 per cent ethyl alcohol. After thorough grinding, the mixture was poured into a 50 cc. centrifuge tube with a narrow neck. Three additional 5 cc. portions of 50 per cent alcohol were added successively to the mortar, ground, and rinsed into the tube, so that the tube contained all the liver to be analyzed. Finally, 20 cc. of petroleum ether were added to the tube; the tube was stoppered and shaken at intervals for 10 minutes. The tube was then centrifuged and the vitamin A determined in the petroleum ether layer by the method described for blood. It is desirable to use such an amount of the petroleum ether extract that the standard in the colorimeter will read between 20 and 30 mm. with the unknown set at 20 mm. The average of three determinations on separate portions of the extract was taken in making the final calculation, which is expressed as the number of blue units per 100 gm. of liver.

We found that the blood and liver of rats fed on the Sherman B diet did not contain significant amounts of the carotinoid pigments. Therefore no corrections for the presence of these pigments were made in the values obtained.

Body fat. The method employed was the same as that described for liver. It was found that the presence of too much fat interfered with the color given in the antimony trichloride

reaction, therefore no more than 2 cc. of the petroleum ether extract was used in preparing the solution for each colorimetric reading.

The above method is that of Carr and Price ('26) with some modifications by S. W. Clausen as yet unpublished.[2]

Experiment 1. Young rats

Six rats, 54 days old were used. Three of them were fed 1 drop of halibut liver oil (Abbott Laboratories, serial no. 25954). The remaining three kept as controls were fed no oil. They were killed 24 hours later and the livers, blood and body fat analyzed for vitamin A. This experiment was a preliminary one, and it should be mentioned that the rats used came from three families that after weaning had developed a spontaneous infection with Pasteurella, which, since it was entirely limited to this group, was probably acquired during transportation. Various other members of these families had died, bacteriological examinations had been made by Prof. George Berry, and we had decided to sacrifice the whole group to prevent spread of infection. The six rats used showed some slight loss of weight at the time the experiment was done, and may have been infected with Pasteurella at the time the oil was given. We have no reason to think that in such a short experiment this possible intercurrent infection had any influence on the results.

[2] "The modifications as described above are: a) the use of a relatively small volume of blood, 2 cc. serum, as compared with 10 to 20 cc. blood used by van Eskelen ('31) and Menken ('32); b) the carrying out of the blood extraction in one step as compared with the methods described by van den Bergh and Muller ('20); c) the selection of 10 per cent copper sulfate as a standard for colorimetric use instead of a Lovibond Tintometer; d) the rapid extraction of vitamin A from liver and fat by simultaneous use of two immiscible solvents, as compared with the more prolonged procedures described by Wilson ('27), Simmonet et al. ('31) and Moore ('30). The dilute alcohol penetrates the tissue, and liberates the vitamin A, which is immediately passed on into the much better solvent, petroleum ether. The latter solvent alone does not readily extract vitamin A or carotinoids from moist tissues. This method has proved to be simple and rapid, and it avoids the danger of the destruction of vitamin A by too vigorous treatment. In criticism of the method it may be said that the blue color is not absolutely characteristic of vitamin A, or altogether proportional to the amount present, because the fatty acids have not been removed by saponification. Whilst fully alive to this criticism, we found such wide differences in our results under varying conditions of experimentation, and such good agreement in the results of the groups treated alike, that we feel the results justify the use of the method." (S.W.C.)

The results are seen in table 1. The oil was given from a dropper in a dark room by the light of a 10 watt green Mazda lamp, and one rat, no. 93, was fed 2 drops in error. It is interesting to note the increased vitamin A in the blood, liver and body fat of this rat. None of these rats showed any infection of the middle ear. In this group the whole livers were not weighed at autopsy; the calculations for vitamin A were based on the amount per 100 gram liver. In order to bring this series into line with our other experiments, where the livers were weighed, we calculated the vitamin A in the whole livers on the basis of the liver weights according to body weight given by Donaldson ('24).

TABLE 1

Young rats, Sherman B diet 54 days old

TREATMENT	NO. RAT	LITTER	SEX	LIVER			BLOOD UNITS VITAMIN A/100 CC.	BODY FAT UNITS VITAMIN A/100 GM.	PUS IN EARS
				Weight, grams[1]	Units vitamin A/100 gm.	Units vitamin A whole (calculated)			
No added oil	89	XVI	♂	4.50	310	13.9	16	0	—
	90	XVI	♂	4.50	325	14.6	14	0	—
	91	XVI	♀	4.50	275	12.4	14	0	—
Average				4.50	303	13.6	14.5	0	
1 drop halibut liver oil 24 hours before death	92	XVI	♀	4.00	2290	91.6	18	63	—
	93[2]	XVII	♂	5.00	6220	301.0	34	138	—
	94	XVII	♂	4.50	2910	130.9	21	79	—
Average nos. 92, 94				4.25	2600	111.2	19.5	71	

[1] Liver weights taken from Donaldson's graph.

[2] Fed 2 drops in error.

Experiment 2. Adult rats

Three litters of rats, twenty-four rats in all, which had been reared on the Sherman B diet, were used. At the age of 174 days they were divided into two groups, carefully selected so that the same litters and sexes were represented in both groups. One group of eleven rats was fed 1 drop of halibut liver oil every week for 13 weeks. The remaining thirteen rats were kept as controls with no addition to the diet. At the age of 264 days these rats were killed and analyses of liver and blood for vitamin A were made. In this experiment the animals were killed 6 days after the administration of the last dose of halibut liver oil. Results are seen in table 2.

Experiment 3. Adult rats, further observations

Five groups of adult rats, which varied in age from 186 to 204 days were fed different doses of halibut liver oil at weekly intervals as in experiment 2, 1 drop per week being fed. These

TABLE 2

Adult rats, Sherman B diet 264 days old

TREATMENT	NO. RAT	LITTER	SEX	LIVER			BLOOD UNITS VITAMIN A/100 CC.	BODY FAT UNITS VITAMIN A/100 GM.	PUS IN EARS
				Weight, grams	Units vitamin A/100 gm.	Units vitamin A whole			
No added oil	69	XIV	♂	8.06	2,180	176	14	15	—
	71	XIV	♂	7.71	2,957	228	10	60	+
	72	XV	♀	5.42	2,940	159	24	25	++
	74	XV	♂	8.36	1,225	102	12	10	—
	81	XIV	♀	5.33	2,630	140	7	20	—
	83	XIV	♀	6.22	5,175	322	14	35	++
	85	XV	♀	5.20	5,625	293	11	65	—
	86	XV	♂	8.30	490	41	18	40	—
	88	XV	♀	6.30	4,880	308	13	25	—
	65	XIII	♀	5.69	2,070	118	102	70	—
	67	XIII	♀	6.39	3,660	234	16	0	—
	76	XIII	♂	8.48	835	71	26	40	+
	79	XIII	♀	5.62	2,280	128	13	80	—
			Average	6.70	2,820	178	14	37	
1 drop halibut liver oil weekly for 13 weeks	70	XIV	♀	5.08	48,500	2462	19	111	+
	73	XV	♀	6.43	26,000	1672	15	115	++
	75	XV	♀	6.25	37,167	2334	10	160	—
	80	XIV	♀	6.27	38,000	2383	8	85	—
	82	XIV	♀	5.75	35,833	2060	20	110	—
	84	XV	♂	7.20	32,667	2354	13	...	++
	87	XV	♂	9.35	24,500	2290	24	...	—
	66	XIII	♂	7.34	19,583	1437	21	140	—
	68	XIII	♂	7.73	34,000	2627	25	65	—
	77	XIII	♀	5.59	31,667	1771	19	180	—
	78	XIII	♀	5.88	37,333	2196	14	95	+
			Average	6.62	33,204	2144	17	118	

Nos. 70 to 78 last drop of oil given 6 days before death.

five groups received a total of 4, 6, 7, 9 and 11 drops, respectively. They were killed at the widely differing intervals of 28, 137, 132, 97 and 64 days after the administration of the last drop of oil. The object of this experiment was to trace, if possible, additional storage in the liver with increased

dosage, and to find out evidence, if any, of a liberation of vitamin A from the liver in adult life. Results are seen in table 3 and figure 1. The vitamin A values in the liver, already given in table 2, are included in table 3 and figure 1 to complete the story.

Experiment 4

A family of seven young rats, whose mother had received 1 drop of halibut liver oil the day before the young were born, and 1 drop per week during the 3 weeks of lactation, were killed at the age of 54 days and analyses for vitamin A in tissues made. Results of this experiment are seen in table 4.

TABLE 3

Adult rats, Sherman B diet

AGE WHEN KILLED (DAYS)	NUMBER OF RATS	TOTAL NUMBER OF DROPS FED	KILLED DAYS AFTER LAST DROP	LIVER—AVERAGE			BLOOD AVERAGE UNITS VITAMIN A/100 CC.	BODY FAT AVERAGE UNITS VITAMIN A/100 GM.
				Weight, grams	Units vitamin A /100 gm.	Units vitamin A whole		
264	13	None	...	6.70	2,820	178	14	37
186	3	4	28	6.98	13,517	946	10	57
203	3	6	137	4.30	27,233	1170	12	23
206	3	7	133	5.48	23,800	1334	13	13
204	5	9	97	4.53	36,423	1619	13	78
200	11	11	64	7.86	21,950	1712	23	147
264	11	13	6	6.62	33,204	2144	17	118

Experiment 5

One drop of the oil used was found by our method of analysis to contain 437 units of vitamin A. We found that measurement by drops from the dropper supplied with the halibut liver oil bottle was accurate to 2 per cent; 1 cc. of the oil being equal to 51 drops. It seemed advisable to determine how much of a large dose could be recovered from the body of rats in terms of units of vitamin A, and also its distribution throughout the body tissues of the rat. Five mother rats were taken, all of whom had been fed a total of 6 drops of halibut liver oil during pregnancy and lactation, whose tissues, therefore, might be considered to be well stored. They were killed approximately 6 weeks after the birth of their young. Three of them were fed no extra halibut liver oil, the other two were fed 4 drops, in one dose, 24 hours before death. Analyses were made of the tissues listed in table 5. The values given are calculated in each case on the basis of the

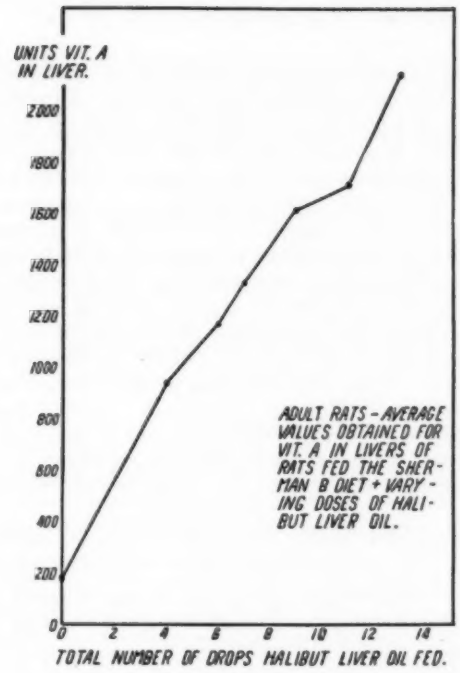

Figure 1

TABLE 4

Young rats, Sherman B diet 54 days old

TREATMENT	NO. RAT	LITTER	SEX	LIVER			BLOOD UNITS VITAMIN A/100 CC.	BODY FAT UNITS VITAMIN A/100 GM.	PUS IN EARS
				Weight, grams	Units vitamin A/100 gm.	Units vitamin A whole			
Mother fed	142	XXII	♂	4.90	560	27.6	23	5	—
a total of 4	143	XXII	♀	3.61	615	22.2	16	41	—
drops hali-	144	XXII	♀	3.45	615	21.2	19	16	—
but liver oil.	145	XXII	♂	4.97	485	24.1	18	14	—
1 drop the	146	XXII	♂	4.93	482	23.7	18	17	—
day before	147	XXII	♂	4.91	484	23.8	14	10	—
birth of	148	XXII	♀	3.97	567	22.6	19	14	—
young, 1 drop weekly during lactation									
			Average	4.40	544	23.5	18	16.7	

weight of the whole organ as weighed at autopsy, with the exception of fat, skeletal muscle and bone. In estimating the total amount of these tissues the figures given by Donaldson were used; viz., 8 gm. for fat, 22 gm. for muscle, and 12 gm. for bone. These are approximate figures for the age and size of the rats. The total amount of blood serum was estimated roughly at 9 cc. The amounts of vitamin A present in these four tissues were so small as to have very little effect on the final result. Therefore we feel that this somewhat rough calculation of the amount of tissue present is justified. In general the technic for analyzing these tissues was similar to that described for liver and fat. For analyses of skin and bone the tissues were frozen with a mixture of acetone and

TABLE 5

Analysis of tissues and organs of female adult rats, for vitamin A content—with and without addition of halibut liver oil, 24 hours before death

RAT NO.	TREATMENT	LIVER UNITS	BLOOD UNITS	FAT UNITS	KIDNEYS UNITS	ADRENALS UNITS	PANCREAS UNITS	SPLEEN UNITS	THYROID UNITS
XXIII	No extra H. O.	1453	1.7	3.8	1.24	0.58	0.01	0.20	0
XXVI		1788	0.9	4.0	0.51	0.91	0.08	0	0.02
XXVIII		1393	1.4	3.1	0.85	0.70	0.09	0.05	0
	Average	1545	1.3	3.6	0.87	0.73	0.06	0.08	0.007
XXIV	4 drops H.O. 24 hours before death	2977	2.7	6.4	1.40	2.10	0.25	0.30	0.05
XXVII		2563	1.8	5.8	2.20	3.30	0.13	0.10	0.02
	Average	2770	2.3	6.1	1.80	2.70	0.19	0.20	0.035

		MUSCLE (SKELETAL) UNITS	LUNGS UNITS	HEART UNITS	BRAIN UNITS	SKIN UNITS	BONE UNITS	INTESTINE UNITS	OVARIES UNITS
XXIII	No extra H. O.	4.8	0.90	0.03	0	9.60	2.6	1.90	0.01
XXVI		1.1	1.02	0.03	0	8.81	3.6	2.23	0.03
XXVIII		8.1	2.21	0.05	0.05	4.27	2.6	0	0.08
	Average	4.7	1.38	0.04	0.02	7.56	2.9	1.38	0.04
XXIV	4 drops H. O. 24 hours before death	11.0	10.80	0.40	0.43	18.90	2.4	40.43	0.14
XXVII		10.7	12.30	0.18	0.09	18.97	4.4	132.99	0.13
	Average	10.8	11.55	0.29	0.26	18.93	3.4	86.71	0.14

Average total units, nos. XXIII, XXVI and XXVIII = 1570
Average total units, nos. XXIV and XXVII = 2915
Total number of units fed, nos. XXIV and XXVII = 1748
Units recovered = 1345
Per cent recovery = 77

solid carbon dioxide in a mortar, a volume of water equal to that of the acetone added, and the mixture extracted with petroleum ether, after this the general technic described for the other tissues was followed. Results of this experiment are seen in table 5.

DISCUSSION OF RESULTS

A comparison of the control rats in tables 1 and 2, which received the Sherman B diet only, shows a constant average level of vitamin A in the blood at 54 days old and at 264 days old. The average value for three rats at 54 days being 14.5 units per 100 cc. and that for thirteen rats at 264 days being 14 units per 100 cc. It is also clear from these control animals that vitamin A is stored with age in the body fat and in the liver. At 54 days no vitamin A was found in the body fat, at 264 days an average value of 37 units per 100 gm. was obtained. At 54 days the average value of vitamin A per 100 gm. liver was 303 units, at 264 days 2820 units. This storage of vitamin A in liver and body tissues, and its constant level in the blood, would indicate that the animal is able to obtain from the diet some vitamin A for storage in adult life. There is still, however, a possibility that the initial requirements in infancy have not been fully met, since the fuller storage of the liver with vitamin A, and its appearance in the body fat, occurs later.

Sherman B diet + added vitamin A. The immediate storage, after 24 hours, of the liver with vitamin A, its appearance in the body fat, and a raised content in the blood is very striking (table 1), especially when we consider rat no. 93 where a double dose of the oil resulted at once in about twice the amount of vitamin A in blood and fat, and more than twice the amount in liver per 100 gm. compared with the values for rats nos. 92 and 94. In the adult rats (table 2) this fact of storage is abundantly confirmed. In this experiment the last dose of oil was given 6 days before the animals were killed. It is interesting to note that the level of vitamin A in the blood of these two groups, with and without the addition of vitamin A is not significantly different. The average values

of the group receiving oil were slightly higher, but the range of values in the two groups was the same. The values for the blood of those receiving oil give no indication of the enormous storage in the liver. This suggests some mechanism whereby the level in the blood is kept constant. The livers of the rats receiving oil contained, on an average twelve times as much vitamin A as those of the rats receiving no oil. The body fat of these rats was also appreciably higher in its vitamin A content. Since 13 drops, over a period of 13 weeks, were given, it seems almost certain that a great proportion of it was stored.

The results of experiment 3 (table 3 and fig. 1) show a definite accumulation of vitamin A, after increased dosage, in the liver of adult rats. Despite the fact that the animals in this experiment were killed at intervals of 28, 137, 97 and 64 days after the administration of the last drop of halibut liver oil, the curve of vitamin A concentration in the liver (fig. 1) steadily rises with doses of 4, 6, 7, 9 and 11 drops. There is no indication that vitamin A was liberated in any appreciable amount from these livers during the experiment.

It is interesting to speculate as to the reason for this striking capacity of the liver to store vitamin A. We think at once of a storage to combat infection, but our results lend no support to the theory of the anti-infective value of vitamin A, when supplied to these rats in adult life. A mild infection of the ears was found in 31 per cent of the animals on the Sherman B diet and in 32 per cent of those receiving added vitamin A (table 2). The addition of vitamin A to the diet of this latter group did nothing to prevent or cure this infection. There still remains the possibility mentioned above that these animals did not receive enough vitamin A in infancy. It is quite possible that the effectiveness of vitamin A to combat infection is determined by the age at which it is given.

Drummond, Coward and Watson ('21) observed that the colostrum of cows was considerably richer in vitamin A than the later milk. These investigators state: "It may indicate

that the child during the first few days of life requires a relatively high concentration of certain substances, amongst them vitamin A, either for its immediate requirements or to provide it with a good reserve at the outset." Similar observations were made later by Luce ('24). These early observations have never been satisfactorily explained. Our results show that the livers of the rats fed on the Sherman B diet only were not stored to capacity with vitamin A, because at 57 days and at 264 days it was possible to raise the vitamin A concentration in the liver by feeding a supplement of this factor. Furthermore, figure 1 indicates that in adult life the livers were capable of storing at least ten times the number of units obtained from the diet only. What the significance of this fuller storage is we do not know, but the fact that the liver has such a great capacity for storage suggests some physiological significance.

When the results of experiment 4 (table 4) are compared with those of rats nos. 89, 90 and 91 (table 1), a definite effect of feeding halibut liver oil to the mother is seen. The average values of vitamin A per 100 gm. liver in rats fed the diet alone was 303 units; that for rats of the same age whose mother had received halibut liver oil, 544 units. In the latter groups higher values were obtained also for blood as compared with those of the first group, and some vitamin A could be detected in the body fat. It remains to be seen, in later experiments, whether this fuller storage of the liver with vitamin A, in early life, results in better initial growth of the rats and protection against the occurrence of spontaneous infection.

In experiment 5 a wide distribution of vitamin A was found in the different tissues examined. Its concentration was surprisingly high in adrenals and lung tissue. The total amount recovered by subtracting that present in the three rats that had received no extra halibut liver oil from those that had been fed the supplement was 77 per cent of the total units fed, and of this 70 per cent was recovered from the liver. In studying the distribution of vitamin A in such a wide range

of tissues, one wonders why certain cells seem to have more of an affinity for vitamin A than others. Further experiments are being planned to elucidate this. It would seem that by our method of analysis a wider distribution of vitamin A in the body has been detected than that observed by other investigators (Moore, '30). It is certainly clear from table 5 that in every case where some vitamin A was found in the organ of the control rats, a definitely larger amount could be detected in the same organ after feeding a large supplement of vitamin A.

CONCLUSIONS

1. By a modification of the Price-Carr method for the determination of vitamin A we have studied the vitamin A content of livers, blood and body fat in young and adult rats fed on the Sherman B diet.

2. Comparing values for vitamin A obtained at 54 days and at 264 days a definite storage of the vitamin A in the liver and body fat was obtained in rats fed the Sherman B diet only.

3. The values obtained at 54 days in the liver are thought to be low, and at this age no vitamin A was found in the body fat. It is suggested that this diet may not contain enough vitamin A to supply the needs of the rat in early life.

4. An immediate storage of vitamin A in the liver and body fat was obtained by feeding 1 drop of halibut liver oil to three young rats 24 hours before death.

5. Eleven adult rats, receiving 1 drop weekly of halibut liver oil for 13 weeks, showed twelve times as much vitamin A in their livers as compared with thirteen control rats from the same litters which received no supplement to the Sherman B diet.

6. The concentration of vitamin A in the blood is no indication of the amount that may be stored in the liver.

7. No effect of vitamin A to prevent or cure the occurrence of a mild infection of the ears in adult rats was observed.

8. Increased storage of vitamin A with increased dosage of halibut liver oil was observed in the livers of adult rats. There was no evidence that this stored vitamin A was liberated from the liver during a period of 28 to 137 days after feeding the vitamin A supplement.

9. By feeding a total of 4 drops of halibut liver oil to a mother rat, 1 drop during pregnancy and 3 drops during lactation, the concentration of vitamin A in the livers of her seven young rats at 54 days old was considerably raised, as compared with that found in three young rats of the same age whose mother had been fed no supplement.

10. Analysis by our method of a number of tissues of the rat for vitamin A showed a wide distribution of this vitamin. We obtained, in terms of units of vitamin A, a recovery of 77 per cent of a large dose of halibut liver oil fed to two rats, as compared with that present in the tissues of three control animals that had been fed no supplement.

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